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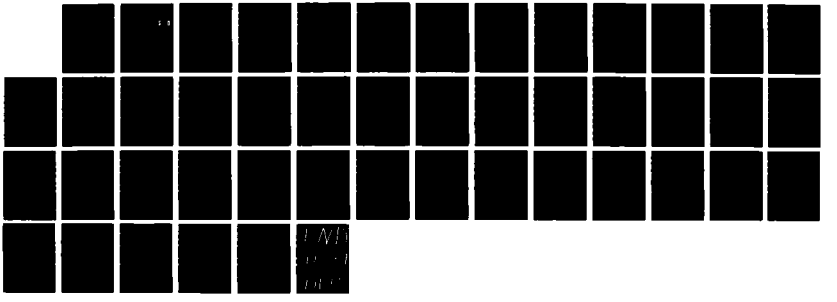

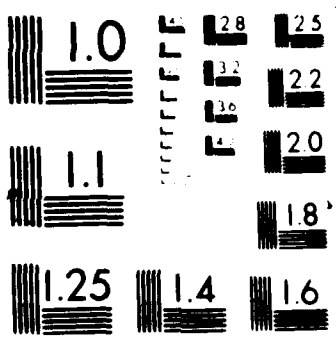
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THE USE OF ATP-$MgCl_2$ IN THE TREATMENT OF INJURY AND SHOCK

Final Report

ARTHUR E. BAUE, M.D.

IRSHAD H. CHAUDRY, Ph.D.

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Studies were conducted to compare the effects of positive end-expiratory pressure (PEEP) and zero end-expiratory pressure (ZEEP) in experimentally induced Pseudomonas pneumonia. Our results indicated a conspicuous advantage of PEEP over ZEEP in experimentally-induced Pseudomonas pneumonia, not only in terms of improved cardiopulmonary function but also in terms of survival. The mechanisms by which PEEP improves the survival of dogs with Pseudomonas pneumonia may be due to an improvement in pulmonary macrophage function.

In studies of circulatory failure, the results indicated that the extent to which ATP levels decrease during shock may be related to the metabolic activity of the organ or tissue. Moreover, membrane-bound sodium-potassium transport mechanism and not membrane permeability is directly affected in shock. In addition, our results indicated that tissue ATP levels are a more sensitive indicator of hypoxia than NAD levels. Various ultrastructural changes (mitochondrial swelling and disruption of mitochondria, increase in

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the number and size of vacuoles, intracellular edema in tissues, absence of glycogen in liver, distortion of endoplasmic reticulum, increasae in lysosomes or other electron dense material, disorganization of brush border in the kidney, clumping or aggregation of material in the area of nuclear membrane in the muscle and endothelial and parenchymal cell swelling) were also found to occur during shock. Studies also indicated that prostaglandins play a protective role during hemorrhage and in early sepsis. The results also lead us to conclude that the changes in RBC cations following shock which have been reported previously by various investigators are not due to shock per se but due to transfusion of stored blood. In addition, basal gluconeogenesis was found to be unaltered during shock and steroids were as effective in stimulating or inhibiting gluconeogenesis during hemorrhagic shock as they were under control conditions. Our studies also demonstrated that insulin resistance which occurs during hemorrhagic shock and can be reversed by treatment of animals in shock with ATP-$MgCl_2$. The results also indicated that ATP can cross the cell membrane of various tissues and this process is enhanced following shock.

Studies of tissue adenine nucleotide levels during sepsis indicated that the changes in adenine nucleotides in late sepsis are similar to those seen during early hemorrhagic shock and suggest inadequate perfusion associated with peritonitis as the cause. In addition, hepatocellular dysfunction occurs even in the early stages of sepsis. The results also indicate that the decreased oxygen consumption observed during sepsis is secondary to decreased oxygen delivery rather than due to a primary defect in oxidative capacity of the hepatocytes. No evidence of insulin resistance was found in sepsis. Treatment of animals in late sepsis with ATP-$MgCl_2$ and glucose was found to significantly improve the reticuloendothelial function (RES) and the survival of animals.

In studies of acute renal failure, our results indicated that infusion of ATP-$MgCl_2$ is effective: 1) in the model of acute renal failure which is not dependent on the initial ischemic episode; 2) when administered 24 hours after the initial insult and 3) in accelerating the course of recovery rather than by simply modifying the severity of the initial insult.

Our results also indicated that treatment of rats with ATP-$MgCl_2$ but not with ATP or $MgCl_2$ alone following total hepatic ischemia, had a salutary effect on the survival of animals. The improved survival was associated with increased hepatic ATP levels, decreased serum enzyme levels, improved RES function and hepatocellular function.

Studies of splenectomy indicated that splenectomy may not only have deleterious effects in terms of host defense systems but also cause prolonged pulmonary changes which may jeopardize the animal as well. Additional studies indicated that autotransplanted splenic tissue improved the RES function and the initial survival rates following splenectomy and sepsis. Our results also indicated that starvation enhances the susceptibility to sepsis. Thermal injury produces alterations in local adenine nucleotide levels. In addition, our studies indicated that transmural potential difference measurements and not muscle contractions provide an extremely sensitive indication of early minimal mucosal damage.

With regards to ATP uptake by tissues, our results indicated that ATP can cross the cel membrane of various tissues and this process is enhanced following hemorrhagic shock. Moreover, the results indicated that the activation of glucose transport by trypsin takes place via the unmasking of the transport system through its proteolytic action while insulin exerts its metabolic effects through membrane conformation alterations.

Studies of the hemodynamic effects of ATP-$MgCl_2$ infusion in anesthetized normal and hypovolemic dogs indicated that ATP-$MgCl_2$ produces positive inotropic, negative chronotropic and peripheral vasodilatory actions, clearly suggesting that this complex has a potential clinical applicability in low flow states.

SUMMARY

Studies were conducted to compare the effects of positive end-expiratory pressure (PEEP) and zero end-expiratory pressure (ZEEP) in experimentally-induced Pseudomonas pneumonia. The results indicated a conspicuous advantage of PEEP over ZEEP in this model not only in terms of improved cardiopulmonary function but also in terms of survival. Moreover, the results suggested that one of the mechanisms by which PEEP improves the survival of dogs with Pseudomonas pneumonia may be due to an improvement in pulmonary macrophage function. The results also suggested that inadequate shuttle enzyme is related to lactate production in the lung and that increased shuttle enzyme activity is associated with alloxan injury.

In studies of circulatory failure the results indicated that the extent to which ATP levels decrease during shock may be related to metabolic activity of the organ or tissue. The results also indicated that ATP generation from tissues of animals in shock was approximately 50% of control. Moreover, membrane-bound sodium-potassium transport mechanism and not membrane permeability is directly affected in shock. In addition, our results indicated that tissue ATP levels are a more sensitive indicator of hypoxia than nicotinamide adenine nucleotide (NAD) levels. Our results also indicated that during severe hemorrhagic shock, various ultrastructural changes occur in tissues. These include mitochondrial swelling and disruption of mitochondria, increase in number and size of vacuoles, intracellular edema in tissues, absence of glycogen in liver, distortion of endoplasmic reticulum, increase in lysosomes or other electron dense material, disorganization of brush border in the kidney, clumping or aggregation of material in the area of nuclear membrane in the muscle and endothelial and paryenchymal cell swelling. Studies also indicated that prostaglandins appear to play a protective role during hemorrhage and also during early sepsis but that other factors may become more important as sepsis evolves. Our results also indicated that hemorrhagic *per se* does not produce any alterations in RBC cations and that the changes which have been reported previously are due to transfusion of stored blood. Moreover, there were no changes in RBC cations during early or late sepsis. Studies also showed that basal gluconeogenesis was unaltered during shock and steroids were as effective in stimulating or inhibiting gluconeogenesis during hemorrhagic shock as they were under control conditions. Our studies have also shown that insulin resistance which occurs during hemorrhagic shock can be reversed by treatment of animals in shock with ATP-$MgCl_2$. In addition, tissue cyclic AMP levels decrease significantly during hemorrhagic shock and the levels are restored following ATP-$MgCl_2$ treatment. Our studies have also demonstrated that ATP is capable of crossing cell membrane of liver, kidney, skeletal muscle and perhaps other tissue cells as well, and that this process is significanly increased in tissues from animals in shock.

Measurement of tissue adenine nucleotides during sepsis indicated that the changes in adenine nucleotides, which are observed in late sepsis, are similar to those seen during early hemorrhagic shock and suggest inadequate perfusion associated with peritonitis as the cause. In addition, our results indicated that hepatocellular dysfunction occurs even in the early stages of sepsis when total hepatic blood flow is increased and that progressive dysfunction occurs in late sepsis concomitant with a decrease in total hepatic blood flow. Moreover, our results support the view that depressed oxygen consumption

A-1

observed in late sepsis is secondary to decreased oxygen delivery rather than due to a primary defect in oxidative capacity of the hepatocytes. In addition, no evidence of insulin resistance in muscles from animals in septic shock was found in our sepsis model of cecal ligation and puncture. Our studies also demonstrated that treatment of animals with ATP-$MgCl_2$ and glucose significantly improved the survival of animals following sepsis. Reticuloendothelial (RES) function was also found to be depressed following sepsis and this was restored following treatment with ATP-$MgCl_2$ and glucose. Although hypertonic glucose administration may play some role in the restoration of RES function, the combined usage of ATP-$MgCl_2$ and glucose was required for complete restoration of RES function following sepsis. We also attempted to decrease the rapidly lethal development of sepsis in our model and this was accomplished by decreasing the number of cecal punctures and the size of the needle for making the punctures.

In studies of acute renal failure in rats, our results indicated that infusion of ATP-$MgCl_2$ after severe renal insult significantly improved both glomerular and tubular function and preserved cellular architecture. Thus, ATP-$MgCl_2$ appears to enhance recovery from severe acute renal injury. Similar results were obtained using mini-pigs. Our results also indicated that cellular recovery of post-ischemic acute renal failure by ATP-$MgCl_2$ could be due to provision of energy to ischemic kidneys, thereby decreasing the severity of the necrotic lesion. Moreover, our results indicated that the accelerated renal recovery by ATP-$MgCl_2$ was concentration-dependent and that optimal effects were observed with 50umoles of ATP and 50umoles of $MgCl_2$. Our results also demonstrated that the infusion of ATP-$MgCl_2$ is effective: 1) in the model of acute renal failure which is not dependent on an initial ischemic episode, 2) when administered 24 hours after the initial insult and 3) in accelerating the course of recovery rather than simply modifying the severity of the initial insult.

Our results also indicated that treatment of rats with ATP-$MgCl_2$ but not with ATP or $MgCl_2$ alone following 60 or 90 min of total hepatic ischemia had a salutary effect on the survival of animals. This beneficial effect could be due to 1) provision of energy directly to hepatocytes; 2) restoration of hepatocyte function, particularly RES function, and 3) restoration of hepatic circulation and prevention of cell swelling.

In studies of splenectomy, the results indicated that splenectomy may not only have deleterious effects in terms of host defense systems but also causes prolonged pulmonary changes which jeopardizes the animal as well. Moreover, our results indicated that splenectomizing the animals prior to cecal ligation and puncture increases the mortality of animals. This leads us to conclude that the spleen plays an important role in the survival of animals following sepsis. In additional studies, splenic tissue was autotransplanted and the results indicated that autotransplanted splenic tissue improved the RES function and initial survival following splenectomy and sepsis.

Our studies also indicated that the activation of glucose transport by trypsin takes place via the unmasking of the transport system through its proteolytic action while insulin exerts its metabolic effect through membrane conformation alterations.

Studies fr[illegible]r laboratory also indicated that starvation enhances the susceptibili[illegible] sepsis and that increased metabolic demand following trauma, couple[illegible] with severe caloric deprivation may have an adverse effect on RES function. Although the precise cause of increased mortality following starvation and sepsis remains unknown, altered hepatic, pulmonary and RES function may play a role.

Additional studies indicated that thermal injury alters local adenine nucleotide levels and is associated with elevated glucose utilization and blood flow in muscles of the burned region. In studies of the ischemic damage to the small intestine, our results indicated that transmural potential difference measurements and not muscle contractions provide an extremely sensitive indication of early minimal mucosal damage. Our studies also indicated that ATP uptake is an active process and that it requires mitochondrially produced ATP for its operation.

Studies of the hemodynamic effects of ATP-$MgCl_2$ infusion in anesthetized normal and hypovolemic dogs indicated that ATP-$MgCl_2$ produces positive inotropic, negative chronotropic and peripheral vasodilatory actions, clearly suggesting that this complex has potential clinical applicability even during hypovolemia.

FOREWORD

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

TABLE OF CONTENTS

TABLE OF CONTENTS (continued). Page

ETC.

Body of Report

Work completed during the period of October 1, 1975 through July 31, 1981 can best be summarized by citing the publications from our laboratory supported by the above contract.

1. Chaudry, I.H., Sayeed, M.M. and Baue, A.E. "Alterations in high-energy phosphates in hemorrhagic shock." Surgery 79:666-668, 1976.

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Reprints of articles published are included for review. We have also participated in a number of programs in which the work supported by this contract has been presented. These include participation and presentation of our work at the Annual American College of Surgeons Meeting, American Association for the Surgery of Trauma Meetings, The Federation Meetings and various other lectures at regional and local programs on shock and circulatory failure.

The principal findings of the above contact are summarized below.

1. LUNG STUDIES

a) THE SALUTARY EFFECTS OF POSITIVE END-EXPIRATORY PRESSURE (PEEP) IN EXPERIMENTALLY-INDUCED PSEUDOMONAS PNEUMONIA.

Since the popularization of positive end-expiratory pressure (PEEP) in the treatment of respiratory distress syndromes, mechanical ventilation with PEEP has become commonly used in management of ventilatory failure in severe pneumonia. However, certain theoretical and practical problems are inherent in the use of PEEP. For example, increase in arterial oxygenation is offset in some patients by a decrease in cardiac index. It also may be questioned whether over-distention of some alveoli and recruitment of others induced by PEEP might disseminate bacteria more widely throughout the lung and impair natural defenses which limit infection. Since these problems have not been systematically examined, a canine model of pneumonia with Pseudomonas aeruginosa was developed to test the hypothesis that PEEP is beneficial in a laboratory preparation ventilated mechanically for 24 hours. We examined the cardiopulmonary response to PEEP in dogs before and after the intratrachael inoculation of Psuedomonas aeruginosa (1×10^9 organisms/kg), thereby allowing us not only to study the cardiopulmonary response to PEEP in the presence of gram-negative pneumonia, but also the effect of PEEP on the course of the experimentally induced pneumonia.

Sixteen mongrel dogs were anesthetized (pentobarbital, pancuronium), intubated and ventilated (16ml/kg and 10 respirations/min) for 24 hours with 50% O_2 and 50% N_2). Half of the animals were maintained with zero end-expiratory pressure (ZEEP) and the remainder had PEEP = 10cm H_2O. Half of the dogs in each group were challenged with live Pseudomonas and the following parameters were measured at 0, 1, 2, 4, 8, 12, 16 and 24 hours: blood pressure, pulse rate, pulmonary artery pressure, pulmonary capillary wedge pressure, respiratory rate, effective compliance minute ventilation, tidal volume, pH, Pa_{O_2}, Pa_{CO_2}, Pv_{O_2}, cardiac ouput, and hematocrit.

Seventy-five percent of infected ZEEP dogs died before 24 hours; all infected animals treated with PEEP and control dogs survived. The infected ZEEP dogs developed a significantly ($p<0.05$) elevated cardiac index as early as 4 hours, accompanied by a significant increase in venous admixture; oxygenation and compliance deteriorated profoundly. Infected ZEEP dogs also showed signs of early capillary leak with a 50% increase in mean hematocrit, despite the infusion of 63% more fluid (13.3ml/kg/hr) than the control group to maintain a pulmonary capillary wedge pressure of 8mmHg. Cardiovascular and respiratory function in the infected PEEP group was not markedly different from the noninfected PEEP or ZEEP control animals.

Tissues for quantitative bacteriology and pathology were obtained at the time of death at 24 hours. The geometrical mean of quantitative bacteria counts from infected-ZEEP lobes was 2.0×10^6 ($\pm$ 3.9) (organisms/g tissue), while the mean of infected PEEP lobes was 1.7×10^4 ($\pm$ 3.6) ($p < 0.05$). Semi-quantitative pathology scores indicated greater

injury to the ZEEP-infected than to the PEEP-infected lungs. Quantitative bacteriology and microscopic evidence of parenchymal injury was positively correlated. Thus, PEEP-treated animals had lower quantitative bacteria counts and less microscopic pulmonary damage.

The above results indicate a conspicuous advantage of PEEP over ZEEP in experimentally induced Pseudomonas pneumonia, not only in terms of improved cardiopulmonary function, but also in terms of survival. The advantage conferred by PEEP may be due to facilitation of local mechanisms of pulmonary defense against infection, to increase systemic resistance to sepsis, or both.

b) AN EFFECT OF POSITIVE END-EXPIRATORY PRESSURE (PEEP) ON PULMONARY ALVEOLAR MACROPHAGE FUNCTION.

The above studies have shown that positive end-expiratory pressure (PEEP) improves survival in a canine model of Pseudomonas pneumonia. Among possible explanations for the observed reduction in mortality, PEEP may improve the function of pulmonary alveolar macrophages (PAMs). This study was therefore conducted to test PAM function after PEEP in an in vitro system for quantitating the uptake of C^{14}-labeled Psuedomonas organisms by PAMs in tissue culture.

Five one-year-old, farm-bred beagle dogs were anesthetized (Pentobarbital) and intubated with a double-lumen Carlen endotrachael tube. The lungs were ventilated synchronously (TV 10 cc/kg x 10 min); and a PEEP valve (10cm H_2O) was used randomly in one circuit or the other so that one lung had PEEP and the other had zero-end-expiratory pressure (ZEEP). After 6 hours the PAMs were lavaged from each lung, quantified, assessed for viability (dye exclusion), and cultured as a monolayer. Pseudomonas organisms were grown in broth containing C^{14}-l-amino acids and also cultured as a monolayer. Viable radioactive organisms were counted, opsonized (IgG antibody) and added to the PAM monolayer for 30 min in a ratio of 100 bacteria per cell. Bacterial uptake was expressed as CPM per lysed monolayer. The results indicated that equal numbers of PAM were obtained from PEEP and ZEEP lungs and the viability index was also equal. Bacterial uptake was linear over the first 45 min by both ZEEP and PEEP PAMs (with $r = .98$ and .99, respectively); but the slope of uptake was increased 40% in the PEEP PAMs. The mean CPM was increased 50% for the PEEP PAMs at 30 min ($p < 0.05$ by paired and unpaired t-test) and 29% at 45 min ($p < 0.05$ by pair t-test).

These results suggest that one of the mechanisms by which PEEP improves the survival of dogs with Psudomonas pneumonia may be due to an improvement in pulmonary macrophage function.

c) LOW LEVELS OF α-GLYCEROPHOSPHATE DEHYDROGENASE IN THE LUNG

Lactate production from carbohydrates under aerobic conditions is a distinct feature of the lung. Similar production of lactate by neoplastic cells has been explained on the basis of inadequate levels of cytoplasmic "shuttle enzymes" required in the oxidation of DPNH, as this promotes lactate production through oxidation of DPNH by lactate dehydrogenase

(LDH). We have measured LDH and the shuttle enzyme α-glycerophosphate dehydrogenase (GPDH) in the lung and liver tissues. These enzymes were also measured in the lung with alloxan-induced injury. Lungs and livers of control rats and lungs of alloxan-treated (IV, 300mg/kg body wt) rats were homogenized and mitochondria free supernatant prepared by differential centrifugation. LDH and GPDH activities in supernatant were assayed by a fluorometric procedure. The results (mean $\pm$ S.E. values of enzyme activities in micromoles DPNH/mg protein/min) for LDH were 261.1 $\pm$ 11.9, 72.6 $\pm$ 5.3 and 132 $\pm$ 14.7 for control liver, control lung and alloxan treated lung respectively. Whereas the GPDH to LDH ratio was 1:1 in liver, it was 1:14 in lung. These data support the concept that inadequate shuttle enzymes is related to lactate production in the lung and that increased shuttle enzyme activity is associated with alloxan injury.

2. CIRCULATORY FAILURE -- ENERGY LEVELS AND MEMBRANE MEDIATED EFFECTS.

a) ALTERATIONS IN HIGH-ENERGY PHOSPHATES IN HEMORRHAGIC SHOCK AS RELATED TO TISSUE AND ORGAN FUNCTION.

Previous work from our laboratory has shown that depletion of liver and kidney ATP levels occur during shock. In contrast to liver and kidney, skeletal muscle did not show any decrease in high-energy phosphate compounds unless the animals were in severe shock. Since the muscle used in the previous study was a resting muscle, the effect of hemorrhagic shock on a working muscle such as the diaphragm remained unknown. To study this, hemorrhagic shock in rats was produced by cannulating the subclavian arteries and bleeding the animals to a mean arterial pressure of 40mmHg. This pressure was then maintained for one hour (early shock) or two hours (late shock). Analysis of the diaphragm (working muscle) showed that there was a significant decrease in ATP, ADP and creatine phosphate levels beginning with early shock, whereas in soleus muscle these changes were not observed until late shock. Moreover, liver and kidney showed greater decreases in ATP levels than the diaphragm during various stages of shock. Thus, in the working organ or tissue, ATP and creatine phosphate levels decrease much earlier than in a resting skeletal muscle during shock. The results also indicate that the extent to which ATP levels decrease during shock may be related to the metabolic activity of the organ or tissue.

b) ATP REGENERATION BY LIVER SLICES IN HEMORRHAGIC SHOCK.

The purpose of this study was to determine whether liver slices from animals in shock were capable of regenerating ATP in vitro. Rats were bled to a mean arterial pressure of 40mmHg and maintained at this pressure until 30% of the shed blood was returned (1 1/2 hours of shock). Animals were then sacrificed with or without reinfusion of the remaining shed blood and liver slices were prepared. Tissue ATP content was measured prior to and after chilling (0.5°C for 90 min) of liver slices in a Krebs-Ringer phosphate (KRP) medium. The slices were then rewarmed (37°C for 60 min) in a KRP and tissue ATP contents were determined. ATP content of chilled and rewarmed liver slices from unbled control animals was measured with and without 1.0mM dinitrophenol (DNP) in KRP medium. The net changes in liver slice ATP (mean $\pm$ S.E. μmoles/g protein) were:

	Control		Shock	
	-DNP	+DNP	Before Reinfusion	After Reinfusion
Preincubation	11.9 ± 0.6	*	3.1 ± 0.8	4.2 ± 0.7
Chilled	6.5 ± 0.4	4.8 ± 0.3	1.9 ± 0.4	3.3 ± 0.5
Rewarmed	11.2 ± 0.6	3.1 ± 0.4	4.0 ± 0.6	5.7 ± 0.8

* No DNP present before incubation

The above data shows that liver slices from both control and shock groups were able to regenerate ATP on rewarming. However, the net increase in tissue ATP from animals in shock was about 50% of control. This approach provides a method for further assessment of tissue energy turnover.

c) HEPATIC Na^+, K^+ TRANSPORT AND CELL MEMBRANE PERMEABILITY IN CIRCULATORY SHOCK.

Cell failure and eventual cell death following decreased tissue perfusion is typical in irreversible circulatory shock. It has been suggested that alterations in membrane transport to electrolytes might play an important role in the pathogenesis of shock. We studied hepatic Na^+ - K^+ transport to determine the relevance of alterations in this function during shock. Rats were subjected to shock by bleeding them to 50% of their total blood volume. The ensuing hypotension (approximately 40mmHg) was maintained for one half, one or two hours. Net Na^+ and K^+ movements were measured with chilling (0.5^o for 90 min) and rewarming (37^o for 60 min) of liver slices in an oxygenated Krebs-Ringer bicarbonate buffer. The magnitude and direction of changes in tissues and intracellular Na^+ or K^+ with chilling was about the same in control and shock animal liver slices. With rewarming, net decreases in Na^+ was 147.8mmoles/kg dry weight per hour in control, 24.4 after a half hour of shock, and not demonstratable in animals after one or two hours after shock. Active K^+ reaccumulation was 139.38 mmoles/kg dry weight in controls, 29.4 after a half hour, 15.8 after one hour and 10.9 after two hours of shock. Replenishment of animal's blood volume restored Na^+ - K^+ transport to near control levels in half hour shock group but not in animals after one hour of shock. Whereas Na^+ - K^+ transport was abolished after one hour of shock, protein synthesis was decreased only to about 50% of control levels. Although the decreased cellular protein synthesis paralleled the decrease in ATP formation by liver slices of animals in shock, the decreased ATP formation could not completely account for the observed failure of Na^+ - K^+ transport in shock.

Although the above studies indicate that hepatic Na^+ - K^+ transport is adversely affected in hemorrhagic shock, it is unclear whether the observed alterations in transport are related to changes in hepatic cell membrane permeability to Na^+ and K^+. We have, therefore, estimated the permeability of liver cells to Na^+, K^+ and chloride of animals in shock and compared it with measurements in control animals' livers. Rats were subjected to shock as described above and the ensuing hypotension (40mmHg) was maintained for one or two hours. Hepatic resting membrane potential was recorded *in situ*. Ion permeabilities were measured with liver slices

which were incubated in a chilled (0.5°C) Krebs-Ringer bicarbonate (KRB) medium. Ion and water content and extracellular inulin space in liver slices were determined following incubations. Plots of rate of penetration of ion into liver cells or rate of ion loss from liver slices against ion concentration gradient allowed estimation of permeability to chloride and K^+. Hepatic permeability to Na^+ could not be estimated in this manner as intracellular Na^+ increased during incubations to levels significantly above the extracellular Na^+. There was no change in chloride and K^+ permeability with shock. Relative permeability of Na^+ and K^+ (P_{Na^+}/P_{K^+}) was also unaffected by shock. Thus, estimates of membrane permeability of Na^+ and K^+ (P_{Na^+}/P_{K^+}) could not provide evidence of overt membrane leaks. These data suggest that membrane bound Na^+-K^+ transport mechanism and not membrane permeability is directly affected in shock.

d) DIFFERENCES IN THE ALTERED ENERGY METABOLISM OF HEMORRHAGIC SHOCK AND HYPOXEMIA.

Although tissue hypoxia has been thought to play a major role in the problems produced by circulatory failure and shock, it has been difficult to establish this and to separate it from other problems produced by decreased tissue perfusion. In studying alterations in energy metabolism with shock and how they may be corrected, it became necessary to determine the role that tissue hypoxia might play in these alterations. We have, therefore, determined the effect of hypoxia per se, anoxia per se and hemorrhagic shock on the levels of pyridine and adenine nucleotides of various tissues. The results indicate that ATP levels in liver and kidney of animals in shock or animals subjected to seven minutes of anoxia decreased by 85% and 73%, respectively. Under hypoxic conditions (arterial P_{O_2} 18mmHg), the decrease was only 62% and 48% in liver and kidney, respectively. Tissue NAD levels decreased and NADH levels increased during shock but were found to be essentially unaltered during experimental hypoxemia. Thus, shock produced greater alterations in adenine and pyridine nucleotides than did hypoxemia alone indicating that stagnant hypoxemia due to shock is more deleterious to energy metabolism than is severe hypoxemia with an otherwise normal circulation. The results also suggest that if an arterial P_{O_2} of 18mmHg represents the initial stages of tissue hypoxia, then tissue ATP levels are a more sensitive indicator of this than NAD levels.

e) ULTRASTRUCTURAL CHANGES DURING CIRCULATORY SHOCK.

The above studies showed that NAD, NADH and the ratio NAD:NADH is preserved during hypoxemia but that during shock there is a decrease in the NAD levels and an increase in the NADH levels. These results would suggest that the metabolic activity of the organ is preserved during hypoxemia but not during hemorrhagic shock. Thus, it is possible that the subcellular morphology is also altered during shock. To determine this, hemorrhagic shock was produced by bleeding the rats to a mean arterial pressure of 40mmHg which was then maintained for two hours. At the end of the shock period, the descending aorta was isolated and cannulated with a PE-90 tubing. The animal was then perfused with a solution containing glutaraldehyde- parafinaldehyde (2.4%) through the descending aorta for 10

min. With this procedure, various tissues and organs are fixed *in situ* within a minute; thus, this procedure is much superior to fixation of tissues *in vitro*.

Preliminary experiments from our laboratory have shown that during severe hemorrhagic shock, ultrastructural changes occur in various tissues and organs. These include:

1. Mitochondrial swelling and some destruction of mitochondria.
2. Increase in number and size of vacuoles.
3. Intracellular edema in tissues.
4. Absence of glycogen in liver.
5. Distortion of endoplasmic reticulum and increase in lysosomes or other electron dense material.
6. Disorganization of brush border in the kidney.
7. Clumping or aggregation of material in the area of nuclear membrane in the muscle, and
8. Endothelial and parenchymal cell swelling.

f) PROSTAGLANDIN (PG) METABOLISM SYNTHESIS AND BLOCKADE IN HEMORRHAGE AND SEPSIS.

Studies of *in vivo* PG metabolism by the lung and the effects of PG synthesis blockade by indomethacin (INDO) on survival following hemorrhage and sepsis have been conducted. Pulmonary PG metabolism was assessed by bolus IV injections of 1.24 Ci ^{3}H-PGE_2 in awake restrained rats, followed immediately by withdrawal of blood from the subclavian artery for 20 seconds. PGE_2 and metabolites were separated using thin layer chromatography and quantified as fractional radioactivity due to each compound. Unhemorrhaged controls (N = 6) showed 79.3% metabolism of injected PGE_2. Animals subjected to 75 minutes of hemorrhage at 40mmHg (N = 9) showed a decrease in PGE_2 metabolism to 42.6% ($p<0.01$). Also noted was a decrease in the formation of 15-keto-PGE_2 (28.9% vs 17.3% of the total recovered radioactivity, $p<0.05$). Whether the decreased metabolism is related to altered transport, decreased degradation of PG or altered pulmonary circulation is not known.

PG levels during shock were measured but were so variable as to make interpretations difficult. In another study, IV INDO (5mg/kg) or buffer was given 20 minutes prior to bleeding and INDO (3.5mg/kg/hr) or buffer infused at 0.01ml/min during the 75 minute intervals of hypotension at 40mmHg. Survival (measured over a period of two days) decreased from 68.8% (11/16) in controls to 29.8% (5/17) following INDO treatment ($p<0.025$). Thus, PGs appear to play a protective role during hemorrhage.

In a model of sepsis in rats produced by cecal ligation and puncture, followed by cecal excision at 10 or 16 hours (early or late sepsis,

respectively), the effect of INDO treatment on survival was studied. INDO treatment comprised: 1mg/kg s.q. twice a day for 72 hrs starting 24 hrs before cecal ligation and puncture; 4mg/kg IP 30 minutes before cecal ligation and puncture and 6.7mg/kg IV at the time of cecal excision. Controls received equal volumes of vehicle. Survival (measured over a period of five days) following early sepsis was 100% (12/12) in controls and 25% (3/12) in INDO treated group ($p<0.001$). Following late sepsis, survival was 30.8% (4/13) in controls and 23.1% (3/13) in the treated group. It is concluded that PGs play a protective role during early sepsis but that other factors may become more important as sepsis evolves.

g) FACTORS AFFECTING RED BLOOD CELL (RBC) SODIUM AND POTASSIUM LEVELS DURING SHOCK.

Since increases in RBC Na^+ have been observed in several pathological states, including uremia, burns, trauma and hemorrhagic shock, we investigated the effects of hemorrhagic shock and corrective therapy with whole blood transfusions on RBC Na^+ and K^+ levels. Rats were bled rapidly to a pressure of 40mmHg following which no further blood was removed or returned (Group 1). The blood pressure of these animals increased to approximately 70mmHg within 30 minutes of hemorrhage and remained at that level for 2 hours. Another group of animals were bled to 40mmHg and maintained at that level for 1-1/2 hours by transfusion with rats' whole blood which had been stored in ACD buffer at 4°C for 6 days and warmed to ambient temperatures prior to administration (Group 2). The volume of the blood transfused per animal equaled 50% of the maximally shed blood volume. The results of eight animals in each group in mM/L were as follows:

	GROUP 1		GROUP 2		STORED BLOOD
	Control	Shock	Control	Shock	
Na^+	3.7 ± 0.1	3.4 ± 0.1	3.5 ± 0.1	8.4 ± 0.7	8.2 ± 1.4
K^+	99.1 ± 1.9	97.2 ± 1.4	100.3 ± 2.3	85.0 ± 2.1	79.4 ± 8.3

These results indicate that hemorrhagic shock without transfusion therapy does not cause any change in RBC Na^+ and K^+ levels. However, reciprocal Na^+ and K^+ alterations in RBC were observed in animals which received ACD, preserved, stored whole blood. In an additional study, transfusion during shock of fresh blood mixed with ACD buffer produced no significant alterations in RBC Na^+ and K^+ levels. Another set of experiments indicated that the increase in RBC Na^+ in Group 2 rats in shock transfused with stored blood for 3 days in ACD was 62%, compared to 140% increase following transfusion of 6 days stored blood. RBC Na^+ of the stored blood itself increased by 75% at 3 days and 127% after 6 days storage at 4°C, probably due to inhibition of the Na^+ pump at lower temperatures. These results indicate that hemorrhagic shock *per se* does not produce any alterations in RBC cations. Because storage of blood at low temperatures causes RBC cations to be altered, this suggests that during shock the changes which have been reported previously are due to transfusion of stored blood.

In another set of experiments, sepsis in rats was produced by cecal ligation and puncture as we have described previously. Measurement of RBC Na^+ and K^+ levels at 10 and 16 hours (early or late sepsis, respectively) following cecal ligation indicated that there were no significant alterations in RBC cations during early or late sepsis as well.

h) EFFECT OF GLUCOCORTICOIDS ON GLUCONEOGENESIS DURING HEMORRHAGIC SHOCK.

This study was undertaken to determine if there was evidence of impaired gluconeogenesis during shock and to test the effectiveness of steroids on gluconeogenesis during such conditions. Holtzman rats were cannulated and bled to a mean arterial pressure of 40mmHg which was maintained for two hours. Control animals were treated in exactly the same manner but were not bled. After decapitation of the animals, liver slices (0.2 to 0.4mm thick, average weight 30mg) were prepared and incubated for three hours at 37°C in Krebs-bicarbonate medium containing no added substrate or 10mM alanine with or without hydrocortisone. Glucose and urea production in the medium was measured following incubation. Hydrocortisone addition at 10^{-7}M inhibited gluconeogenesis by 30% whereas with 10^{-2}M hydrocortisone there was a complete inhibition of gluconeogenesis with both groups of slices. Similar results were obtained using dexamethasone or hydrocortisone-21 sodium succinate. Thus, basal gluconeogenesis was unaltered during shock and steroids were as effective in stimulating or inhibiting gluconeogenesis during hemorrhagic shock as they were under control conditions.

i) INSULIN RESISTANCE AND ITS REVERSAL IN VITRO ADMINISTRATION OF ATP IN HEMORRHAGIC SHOCK.

Hemorrhagic shock was produced by bleeding rats to a mean arterial pressure of 40mmHg which was maintained for two hours. Muscles from these animals ('shock muscles') showed resistance to the stimulation of glucose uptake by insulin. Addition of 1mM ATP-$MgCl_2$ to the medium had no effect on basal glucose uptake in either group of muscles, but it permitted insulin to exert its stimulatory effect in shock muscles. An optimal insulin effect on glucose uptake in shock muscles was observed at a concentration of 0.007 U/ml. Increasing the concentration of ATP-$MgCl_2$ to 2.5mM in the medium resulted in an optimal insulin effect at an insulin concentration of 0.001 U/ml in shock muscles. Following one hour of incubation in Krebs-bicarbonate medium, intracellular ATP contents of shock muscles were approximately 50% lower than in control muscles. Addition of 1mM ATP-$MgCl_2$ to the incubation medium had no effect on the intracellular ATP contents of either group of muscles following incubation. However, 2.5mM ATP-$MgCl_2$ elevated intracellular ATP contents of shock muscles but had no effect on control muscle. We have previously proposed that the insulin resistance in tissues from animals in shock could be due to a change in the membrane conformation. Whether the effect of ATP-$MgCl_2$ is due to reversal of cellular swelling or due to some other membrane or metabolic effect is not known at the present time.

j) REVERSAL OF INSULIN RESISTANCE BY IN VIVO INFUSION OF ATP IN HEMORRHAGIC SHOCK.

Having found that addition of ATP-$MgCl_2$ to the medium containing muscles from animals in shock reversed the tissue insulin resistance, we then considered the possibility of infusion of ATP-$MgCl_2$ into animals in shock to investigate whether this would reverse the insulin resistance seen in vivo during shock. Following two hours of hypotension at 40mmHg, ATP-$MgCl_2$, ADP-$MgCl_2$, adenosine-$MgCl_2$ or GTP-$MgCl_2$ (25 μmoles/25ml) was infused into animals followed by the return of the remaining shed blood. Thirty minutes following the return of the shed blood, the animals were sacrificed. Muscles were then removed and the effect of insulin on glucose uptake in these muscles was studied. Infusion of ATP-$MgCl_2$, ADP-$MgCl_2$, adenosine-$MgCl_2$ or GTP-$MgCl_2$ into animals following shock had no effect on basal glucose uptake; however, treatment with ATP-$MgCl_2$ but not with the other nucleotides permitted insulin to exert its stimulatory effect on such muscles. An optimal insulin effect in ATP-$MgCl_2$ treated shock muscle occurred at an insulin concentration of 0.001 U/ml which is also the concentration required to produce optimal insulin effect in control muscles. Following one hour of incubation in Krebs-bicarbonate medium, ATP contents of shock muscles were approximately 50% lower than in control muscles. Treatment with ATP-$MgCl_2$ following shock, however, resulted in ATP contents in such muscles similar to those in control muscles. The exact mechanism for the reversal of insulin resistance by ATP-$MgCl_2$ infusion in shock is not known at present.

It has been shown that in humans, severe injury and shock are associated with hyperglycemia and an abnormality in glucose tolerance that persists after the injury. Although the present results indicate that insulin resistance during shock could be overcome by administration of ATP-$MgCl_2$, a strong caveat regarding hasty clinical application at the present stage is appropriate. Further experiments on the mechanism of this reversal and the possible side effects of such infusions are needed.

k) ALTERATIONS IN ADENOSINE 3'-5'- MONOPHOSPHATE LEVELS IN HEMORRHAGIC SHOCK.

Adenosine 3'-5'- monophosphate (CAMP) has been shown to regulate cell function and to mediate physiologic responses of cells to many stimuli. If the effectiveness of the CAMP system is decreased, this could result in loss of control of certain vital cellular activities. Since the formation of CAMP is through ATP, and since our previous work has shown that ATP levels were significantly reduced during hemorrhagic shock, it seems reasonable to expect that the CAMP system might also be altered during shock. To determine this, Albino Holtzman rats were cannulated, bled and maintained at a pressure of 40mmHg for two hours. Following sacrifice, small pieces of liver, kidney, muscle and brain were quickly removed and frozen in liquid nitrogen. Tissues were then homogenized and CAMP levels measured by the radioimmunoassay procedure. The results indicate that there are significant decreases in liver, kidney, muscle and brain cyclic AMP levels in shock. We have previously shown that with the same period of shock ATP levels in liver, kidney and muscle decreased. The decreases in CAMP levels follow the same trend as the decreases in ATP levels suggesting

that these events are related. The precise mechanism for decreased CAMP levels is not known at the present, however, it is possible that this is due to decreased ATP levels within the cell during shock.

l) RESTORATION OF CELLULAR ADENOSINE 3'-5'-MONOPHOSPHATE LEVELS BY ATP-$MgCl_2$ IN HEMORRHAGIC SHOCK.

Previous work has shown that ATP and adenosine 3'-5'-monophosphate (CAMP) levels in various tissues decrease during shock and that treatment of animals in shock with ATP-$MgCl_2$ restores the tissue ATP levels. Since the formation of CAMP is through ATP, CAMP levels might also be affected by ATP-$MgCl_2$ treatment. In order to test this, fasted Holtzman rats were bled to a pressure of 40mmHg and maintained for two hours. At the end of the shock period, ATP-$MgCl_2$ (0.25ml/25μmoles each) or saline (0.25ml) was infused intravenously along with the return of the remaining shed blood. Thirty minutes after the above treatment, animals were sacrificed and small pieces of liver, kidney, muscle and brain were quickly removed and frozen in liquid N_2. A protein-free extract of tissues was prepared and CAMP was measured by the radioimmunoassay procedure. The levels of CAMP (picomoles/gm; mean + SEM; n = 8 for each value) in liver, kidney, muscle and brain were: 1242 + 88, 1602 + 97, 917 + 70 and 3785 + 470, respectively. During shock, CAMP levels in the above tissues decreased to 482 + 36, 627 + 97, 676 + 57 and 2289 + 264, respectively, and these values did not change following blood or saline infusion. CAMP levels of animals treated with ATP-$MgCl_2$ and blood following shock increased to 1195 + 90, 1710 + 100, 1186 + 84 and 4062 + 490, respectively. The precise mechanism for the restoration of CAMP levels following shock by ATP-$MgCl_2$ treatment is not known at present.

m) UPTAKE OF ATP BY LIVER AND KIDNEY IN VITRO.

Cell membranes are believed to be impermeable to ATP but many experimental observations indicate the contrary. A number of studies have shown that ATP is released from active skeletal muscle under conditions in which potassium is not. More recently, it has been shown that ATP is released from motor nerve terminal on indirect stimulation of mammalian nerve-muscle preparation. We have previously shown that ATP can enter intact skeletal muscle cells. From these studies, it would appear that the release and uptake of ATP is a physiological process. This being the case, the process should also be present in tissues such as liver and kidney. In order to study this, rats were decapitated following which liver and kidney were quickly removed and tissues divided into small blocks (about $5mm^3$). Slices from liver and kidney (0.3 to 0.5mm thick, average weight 40mg) were then prepared from the tissue blocks with razor blades within 5 to 8 minutes after excision. Two to five slices from each organ were incubated for 1 hour at 37°C in 1.0ml of Krebs-Henseliet bicarbonate buffer (pH 7.4) containing 10mM glucose, 5mM $MgCl_2$ and one of the following: 5mM [8-^{14}C] ATP (0.45 uC/umole); 5mM [8-^{14}C] ADP (0.25 uC/umole); 5mM [8-^{14}C] AMP (0.25 uC/umole) or 5mM [8-^{14}C] adenosine (2 uC/umole) under an atmosphere of 95% oxygen, 5% CO_2 in each case. At the end of the incubation period, slices were removed, rinsed quickly in ice-cold water, blotted on a dampened filter paper and frozen between two aluminum blocks chilled in dry ice. The slices were then homogenized in 1.0 ml of a solution containing trichloracetic acid (10%) and HCl (0.1 N) and centrifuged.

The supernatant solution was extracted four times with water-saturated ether and then neutralized with 1.0 M Tris base. Samples (50ul) of tissue extract and incubation medium were applied to a Whatman No. 3MM paper and subjected to electrophoresis. Following electrophoresis separation, radioactivity in the individual nucleotide spots was counted in a scintillation counter. The concentration of adenine and hypoxanthine nucleotides in medium (μmoles/ml) and tissues (μmoles/g) was calculated from the radioactivity observed in each fraction. A nucleotide was considered to have an intracellular distribution when the total tissue content exceeded the extracellular content. Extracellular concentrations were calculated on the assumption that the concentration in the extracellular water was the same as that of the medium.

Extensive degradation of the added nucleotide was observed in the presence of both liver and kidney. The concentrations of ^{14}C-ATP and ADP found in the liver and kidney indicated that these compounds were present within the cells. If the medium ATP levels at the end of the incubation period were plotted, it became apparent that by maintaining higher medium ATP levels intracellular ATP increased in both liver and kidney. The relationship between medium ATP levels and intracellular ATP i.e., ATP uptake, however, was not linear. There was approximately 100% increase in intracellular ATP when medium ATP levels were maintained at levels ranging from 0.18 μmoles/ml to 1.85 μmoles/ml. By further maintaining the medium ATP at levels extending to 3.5 umoles/ml, intracellular ATP increased by 45% only. These results suggest that ^{14}C-ATP uptake activity was at a near saturation level when medium ATP levels were maintained at 1 μmole/ml. Since the occurrence of saturation in uptake activity with increasing medium substrate concentration indicates the involvement of a membrane carrier in the uptake process, the results presented above suggest that ATP uptake in liver and kidney could be a carrier mediated process. The intracellular concentration of ATP in liver and kidney exceeds that in the medium at the lowest concentration tested (0.18 umoles). Whether this suggests the existence of a low capacity active transport system for ATP is not known at present. These results indicate that liver and kidney cells are permeable to ATP and to a lesser extent to ADP and they suggest that the ATP uptake process in these organs could be a carrier mediated process. Previous work has shown that ATP can cross intact skeletal muscle cells. Thus, it would appear that ATP is capable of crossing cell membranes of liver, kidney, skeletal muscle and perhaps other tissue cells as well.

n) EVIDENCE FOR ATP TRANSPORT INTO CELLS.

Work from our laboratory has shown that ^{14}C-labeled ATP can enter the intact cell of muscle, liver and kidney and that this may be a carrier mediated process. However, the subcellular distribution of ATP within the preparation into which the nucleotides entered is not known at the present time. In order to demonstrate beyond doubt that ATP can penetrate the cell membrane, we have studied the uptake of ^{32}P- and ^{14}C-labeled ATP mixture. Rats were decapitated following which two soleus muscles from each rat were quickly removed and incubated for one hour at 37^{o}C in 1.0ml of Krebs-Henseliet bicarbonate buffer (pH 7.4) containing 10mM glucose, 5mM $MgCl_2$, 2.5mM [8-^{14}C] ATP (0.45 μCi/umole) and 2.5mM [^{32}P] ATP (0.45

μCi/umole) under an atmosphere of 95% O_2: 5% CO_2. At the end of the incubation period, muscles were removed, rinsed quickly in ice-cold water, blotted on a dampened filter paper and frozen between aluminum blocks chilled in dry ice. The muscles were then homogenized in 1.0 ml of a solution containing trichloracetic acid (10%) and HCl (0.1 M) and centrifuged. The supernatant solution was extracted four times with water-saturated ether, neutralized with 1.0 M Tris base and subjected to electrophoresis. Following electrophoretic separation, ^{32}P and ^{14}C radioactivity in the individual nucleotide spots was counted in a scintillation counter.

The results incidated that extensive degradation of ^{14}C-ATP and ^{32}P-ATP was observed in the presence of muscles. The concentrations of ^{14}C-ATP and ^{32}P-ATP found in the muscle indicated that these compounds were present in equal concentrations within the cells. Since both ^{14}C-ATP and ^{32}P-ATP entered the muscle cell in equal amounts, this clearly indicates that the cell membrane is permeable to ATP.

o) EVIDENCE FOR ENHANCED UPTAKE OF ATP BY LIVER AND KIDNEY IN HEMORRHAGIC SHOCK.

It has been shown that infusion of ATP-$MgCl_2$ proved beneficial in the treatment of shock; however, it is not known whether this effect is due to improvement in the microcirculation or direct provision of energy or a combination of the above or other effects. To elucidate the mechanism of a salutary effect of ATP-$MgCl_2$, we examined the in vitro uptake of ATP by liver and kidney of animals in shock. Rats were bled to a mean arterial blood pressure of 40mmHg and maintained at that pressure for two hours. Following sacrifice, liver and kidney were removed and slices of tissue (0.3 - 0.5mm thick) were incubated for one hour in 1.0 ml of Krebs-HCO_3 buffer containing 10mM glucose, 5mM $MgCl_2$ and 5mM [8-^{14}C] adenosine in 95% O_2 - 5% CO_2 and then homogenized. Tissue and medium samples were subjected to electrophoresis to separate and measure the various nucleotides. The uptake of ^{14}C-ATP but not that of ^{14}C-ADP or ^{14}C-adenosine by liver and kidney slices from animals in shock was 2.5 times greater than the corresponding uptake by control slices. Thus, the beneficial effect of ATP-$MgCl_2$ in shock could be due to provision of energy directly to tissue in which ATP levels were lowered.

3. STUDIES WITH SEPSIS

a) ALTERATIONS IN TISSUE ADENINE NUCLEOTIDES DURING SEPSIS.

Tissue adenine nucleotide levels were measured in rats to determine if there is a depletion of energy stores associated with sepsis. Peritonitis was produced by cecal ligation and cecal puncture. At 16-24 hours following ligation, rats which were lethargic but still normotensive (late sepsis) and showed clinical and laboratory confirmation of peritonitis-sepsis were sacrificed and small pieces of tissues were removed and frozen. Adenine nucleotides were measured enzymatically. In late septic rats, ATP levels (mean of 8 animals in each group in μmoles/gm ± SEM) decreased from 1.99 ± 0.13 to 0.71 ± 0.07 in liver and 1.23 ± 0.11 to 0.77 ± 0.09 in kidney cortex. Adenine nucleotide levels from both resting

(gastrocnemius) and working (diaphragm) muscles were unchanged during sepsis. Hydrogen polarograph measurements of hepatic blood flow indicated that flow was markedly decreased at this stage of peritonitis. A second group of rats was prepared in the same manner except that they were sacrificed 10 hours after ligation (early sepsis). Most rats at this stage of sepsis appeared only mildly ill; however, blood cultures obtained from six rats so prepared were all positive. These rats did not show any decrease in either hepatic blood flow or tissue adenine nucleotides. Thus, the changes in adenine nucleotides observed in late sepsis (low-flow septic rats) are similar to those seen during early hemorrhagic shock and suggest inadequate perfusion associated with peritonitis as the cause.

b) ALTERATIONS IN HEPATIC FUNCTION DURING LATE SEPSIS.

This study was undertaken to determine whether functional alterations in the liver occur during sepsis. Sepsis in fasted (24 hours) Holtzman rats was produced by cecal ligation and puncture. Saline (3ml/100gm B.W.) was given subcutaneously at that time. Sixteen hours later, the gangrenous cecum was removed; the peritoneal cavity irrigated with warm saline and the abdominal incision closed. Three hours following cecal removal, blood was obtained from the descending aorta and the serum separated. The results (IU/ml, mean + S.E. of eight animals in each group) indicated that during sepsis serum GOT increased from 37 + 2.4 to 132 + 14.5, GPT 12 + 1.9 to 42 + 3.4 and alkaline phosphatase 33 + 1.9 to 69 + 6.1. Blood glucose levels decreased from 125mg% to approximately 30mg%. Since the level of the above enzymes increased and since severe hypoglycemia was observed, it suggests that liver function may be impaired during sepsis.

c) HEPATOCELLULAR DYSFUNCTION IN EARLY SEPSIS.

Although it is known that hepatic failure occurs in late sepsis, it is not known whether there are alterations in hepatocellular function in the hyperdynamic, hyperglycemic and hyperinsulinemic phase of early sepsis. To study this, indocyanine green (ICG) clearance and serum levels of hepatic enzymes were measured during early and late sepsis. Sepsis in rats was produced by cecal ligation and puncture (CLP). Ten hrs later (early sepsis) total hepatic blood flow (THBF) increased from 23.9 + 1.1 to 30.6 + 1.4 (ml/min/100gm) as measured by H_2 polarography. ICG (5mg/kg B.W.) was then given IV and blood samples taken 5, 6, 8, 10, 12, 15, 18 and 20 min thereafter to determine ICG concentrations. ICG half times (t/2) were 4.99 + 0.15 and 6.57 + 0.51 min for sham-operated and CLP rats, respectively (mean + S.E. n = 16). SGOT and SGPT levels (IU/ml) increased from 38.1 + 0.6 to 69.8 + 2.6 and 9.9 + 0.4 to 25.6 + 1.5 ($p < 0.001$), respectively. Thus, the t/2 of ICG as well as serum enzymes increased significantly during early sepsis. Eight additional rats underwent CLP and were tested 16 hrs later (late sepsis). THBF in late sepsis decreased to 15.5 + 0.5ml/min/100gm. ICG t/2 at that time was 8.2 + 0.48 min and SGOT and SGPT levels were 132 + 14.5 and 42 + 3.4, respectively. In contrast with previous studies using low ICG doses (0.5 mg/kg) at which clearance largely reflects THBF, the present study measures clearance of high dose ICG which is a more specific and sensitive indicator of hepatic function. Thus, the present results indicate that hepatocellular dysfunction occurs even in the early stages of sepsis when THBF is increased. Progressive dysfunction occurs in late sepsis concomitant with a decrease in THBF.

d) HEPATIC O_2 CONSUMPTION IN LATE SEPSIS.

It has been shown that mitochondria isolated from livers of septic rats display an oxidative phosphorylation capacity similar to that of mitochondria from normal hepatocytes. Mitochondrial isolation, however, eliminates any possible influence of alterations in the cytoplasmic environment of the mitochondria during sepsis. The purpose of this study was to determine whether sepsis causes changes at the level of the whole cell that result in depressed hepatic oxidative capacity. Sepsis in rats ($n = 17$) was produced by cecal ligation and puncture (CLP). Sixteen to nineteen hrs after CLP (late sepsis), rats were sacrificed and the liver removed and placed in chilled Krebs-Ringer's phosphate buffer. Slices less than 1mm thick were prepared, weighed and placed in the well of an oxygen monitor with 4ml of buffer containing 10mM glucose, pH 7.4 at 37°C and equilibrated with either 100% O_2 (high O_2) or room air (low O_2). At high buffer O_2 tensions, slices from both control and septic animals exhibited high initial respiratory rates: 31.5 + 2.5 and 34.3 + 3.4$\mu l/min/gm$, respectively (mean + S.E.) ($p < 0.1$). In both groups, the initial high rates of O_2 consumption declined rapidly. This decline was not corrected by increasing buffer tension. At low O_2, initial respiratory rates were approximately 30% of the high O_2 values but did not show the rapid decline observed in high O_2. This may indicate some toxic effect of high O_2 tensions. O_2 consumption in slices from control and septic rats in low O_2 were also not significantly different (12.8 + 1.1 and 14.0 + 0.8, respectively). These results support the view that depressed O_2 consumption observed in late sepsis is secondary to decreased O_2 delivery rather than a primary defect in oxidative capacity of the hepatocytes.

e) STUDIES OF PERIPHERAL GLUCOSE UPTAKE DURING SEPSIS.

Both persistent hepatic gluconeogenesis and peripheral insulin resistance have been postulated as contributing causes to the glucose intolerance associated with sepsis, however, a clear demonstration of insulin resistance has not been provided. The purpose of this study was to determine whether there is insulin resistance at the tissue level and whether basal glucose uptake by muscle is affected during sepsis. Under ether anesthesia, peritonitis was produced in rats by ligation and puncture of the cecum. On the day following cecal ligation and puncture, rats which showed clinical confirmation of peritonitis-sepsis were studied. Following sacrifice and blood collection, the intact soleus muscles from control and septic rats were removed and incubated at 37°C for one hour in 1.0 ml of Krebs-Henseleit bicarbonate buffer containing 10mM glucose in the presence and absence of varying concentrations of insulin. Glucose uptake was measured by the disappearance of glucose from the incubation medium. The results indicate that the serum glucose and insulin levels of early septic (10 hrs following ligation) rats were both higher than in control rats; late septic (16-18 hrs following ligation) rats had depressed serum glucose and insulin levels. Basal glucose uptake (μmoles/g/hr) by muscles from late septic animals showed significant increases over controls when these muscles were incubated in an aerobic environment (11.5 + 1.5 vs 7.2 + 1.3), however, no difference in glucose uptake was observed following anaerobic incubations (15.4 + 0.9 vs 14.7 + 1.0). This would suggest that the

increased basal glucose uptake observed above was due to the tissue already being anoxic or hypoxic. Insulin stimulated glucose uptake in muscles from early septic, late septic and control rats to the same degree. Even at insulin concentrations which produced submaximal stimulation of glucose intake, no evidence of insulin resistance in septic muscles was found with this model.

f) BENEFICIAL EFFECT OF ATP-$MgCl_2$-GLUCOSE ADMINISTRATION ON SURVIVAL FOLLOWING SEPSIS.

Infection remains a serious problem in patients after severe injuries or major life threatening operations. Most such patients who do not survive, die ultimately of multiple, sequential or progressive systems failure. It is now clear that there is a critical ingredient in producing organ failure in sepsis. In fact, remote organ failure is often the first sign of peritonitis or other obscure septic processes. A common cause of pulmonary and renal failure is infection. The reasons why and how a septic process produces organ failure and altered metabolism are not clear. Although appropriate antibiotics, surgical drainage and circulatory support remain the mainstays of treatment, more precise support might be provided if the pathophysiology of sepsis was further clarified.

Since previous studies have shown a salutary effect of ATP-$MgCl_2$ administration on survival of animals following hemorrhagic shock, this study was undertaken to determine if ATP-$MgCl_2$ would improve survival of animals with sepsis. Peritonitis in fasted (24 hrs) Holtzman rats (300-350gm) was produced by cecal ligation and puncture. Saline (3ml/100/gm B.W.) was given subcutaneously at that time. Although all rats were not cultured, blood cultures from eight rats so prepared were all positive for E. Coli, Streptococcus Bovis, Proteus Mirabilis, Enterococcus and Bacteroides Fragilis within six hours. Sixteen hrs later, the peritoneal cavity was reopened, the gangrenous cecum was removed, and peritoneal cavity was irrigated with warm saline and the abdomen was closed in layers. Saline was given subcutaneously again at 8 and 24 hrs (1.5 and 2.5ml/100gm B.W., respectively) after each cecal removal. Glucose levels in blood were found to be approximately 30mg% at the time of cecal removal. (Blood glucose levels in rats fasted for 24 hrs were found to be 130mg%). After measuring blood pressure, animals which were normotensive received intravenously either: a) 0.75ml of ATP (100μmoles) - $MgCl_2$ (50μmoles) and 3ml of saline, b) ATP-$MgCl_2$ + 2ml of glucose (50%) + 1ml of saline (ATP-$MgCl_2$ + glucose), c) 3.75 ml of saline (controls) or d) 2ml of glucose (50%) + 2ml saline, and survival was then measured over a period of five days. The survival rate in rats receiving saline, glucose or ATP-$MgCl_2$ alone was 45% (9/20), 20% (2/10) and 30% (3/10), respectively. Infusion of ATP-$MgCl_2$ + glucose, however, resulted in an 80% (16/20) survival ($p < 0.025$ compared to controls). In another group of animals, tissue ATP levels were measured three hours following cecal removal. Hepatic and renal cellular ATP levels (μmoles/gm) were 1.49 ± 0.08 and 0.73 ± 0.10 ($n = 8$) in the saline-treated group and 2.14 ± 0.18 and 1.27 ± 0.10 ($n = 8$) in the ATP-$MgCl_2$ + glucose treated animals ($p < 0.005$ for both organs), respectively (sham-operated liver and kidney ATP was 1.99 ± 0.13 and 1.23 ± 0.11, respectively, ($n = 10$). Thus, increased survival following sepsis is associated with restoration of cellular ATP

levels which followed ATP-$MgCl_2$ + glucose administration. The precise mechanism of this synergistic effect of ATP-$MgCl_2$ combined with glucose on survival is not clear. The septic hypoglycemic animal may require glucose specifically with the added effect that ATP-$MgCl_2$ provides. The restoration of hepatic and renal ATP levels following ATP-$MgCl_2$ + glucose infusion could beneficially affect organ function and therefore the survival of animals. Lower doses of ATP (less than 100μmoles) with glucose were not effective in other animals in this study. Thus, extirpation of the lesion producing the septic process and metabolic support proved helpful without antibiotic treatment.

g) RETICULOENDOTHELIAL SYSTEM (RES) FUNCTION DURING SEPSIS AND THE EFFECT OF ATP-$MgCl_2$-GLUCOSE ADMINISTRATION ON IT.

Cellular immunity and non-specific host defenses have important influences on infection and survival following severe injury and major operations. The removal of the bacteria from the blood is normally a function of RES, and the main sites of the RES intravascular activity are the liver and spleen, which comprise about 85% and 10% of the total body activity, respectively. Assessment of the functional integrity of the RES following various pathophysiological conditions therefore seems important. Depression of RES function has been found following hemorrhagic shock, burn injury, hepatic ischemia, traumatic shock, abdominal injury and endotoxin shock. Whether or not RES function is depressed during peritonitis is not known.

The studies described above indicated that IV infusion of ATP-$MgCl_2$ + glucose (but not glucose alone or ATP-$MgCl_2$ alone) following sepsis had a salutary effect on the survival of animals. To determine whether the RES function is affected during peritonitis and whether ATP-$MgCl_2$ + glucose has any effect on it, peritonitis in fasted Holtzman rats was produced by cecal ligation and cecal puncture. Saline (3cc/100gm B.W.) was given subcutaneously following cecal ligation and puncture, the peritoneal cavity was reopened and the gangrenous cecum was removed; the peritoneal cavity was irrigated with warm saline and the abdomen was closed in layers. After measuring blood pressure, animals which were normotensive received IV either 3ml saline (non-treated rats) or 0.75ml of ATP-$MgCl_2$ (100μmoles of ATP and 50μmoles of $MgCl_2$) and 2.25ml of 50% glucose (treated animals). Two hours following the removal of the cecum, RES function was elavated by measuring the intravascular clearance of a ^{131}I-trolein labeled gelatinized test lipid emulsion.

The results indicate that the intravascular half-time (t/2) (mean ± S.E. of 8 animals in each group) in control, non-treated and ATP-$MgCl_2$ + glucose treated animals was 7.6 ± 0.6, 12.7 ± 1.8 and 7.1 ± 0.3 minutes, respectively. Since the t/2 in the non-treated animals was approximately doubled ($p < 0.02$) as compared to controls, it indicates that significant depression in RES function occurred during sepsis. Administration of ATP-$MgCl_2$ + glucose following sepsis resulted in the t/2 values similar to sham-operated animals indicating that the impairment of phagocytic activity of the RES was reversed with treatment. While the present studies do not define the specific mechanism of the RES depression during peritonitis, the observation that RES depression was reversed by

ATP-$MgCl_2$ + glucose treatment is quite significant. The potential for administration of ATP-$MgCl_2$ + glucose as passive therapy at a time of marked RES depression following sepsis suggests a new therapeutic modality in the treatment of peritonitis.

h) EFFECT OF HYPERTONIC SOLUTION ON RES FUNCTION DURING SEPSIS.

The above studies showed that RES function was depressed during peritonitis and that administration of ATP-$MgCl_2$ + 50% glucose restored the RES function. Since the treatment solution was hypertonic and hypertonicity may produce a transient effect on blood flow which may affect the recovery of the RES function, further studies were conducted to determine the role of hypertonicity on the RES function. Peritonitis in fasted rats was produced by cecal ligation and cecal puncture. Saline (3cc/100gm B.W.) was given subcutaneously following cecal ligation and puncture. Sixteen hours following cecal ligation and puncture, the peritoneal cavity was reopened and the gangrenous cecum was removed; the peritoneal cavity was irrigated with warm saline and the abdomen was closed in layers. After measuring blood pressure, animals which were normotensive received intravenously either: 1) 3ml of 50% mannitol; 2) 3ml of 50% glucose; 3) 3ml saline or 4) 0.75ml ATP-$MgCl_2$ (100 moles + 50 moles) + 2.25ml saline. Two hours following the removal of the cecum, RES function was evaluated by measuring the intravascular clearance of a ^{131}I-triolein-labeled gelatinized test lipid emulsion.

The intravascular half time (t/2) (mean $\pm$ S.E. of 8 animals in each group) in control, mannitol-treated and glucose-treated animals was 7.6 $\pm$ 0.6, 13.5 $\pm$ 1.3 and 9.9 + 0.04, respectively. The t/2 in saline-treated (non-treated) or ATP-$MgCl_2$ alone-treated animals was 12.7 $\pm$ 1.8 and 9.8 $\pm$ 0.5, respectively. Since the t/2 in the mannitol-treated animals was approximately doubled ($p<0.02$) as compared to controls, it indicates that infusion of hypertonic mannitol had no significant effect on the restoration of RES function. Infusion of glucose alone or ATP-$MgCl_2$ alone, however, decreased the t/2 but these values were still much higher than controls or the group that received ATP-$MgCl_2$ + glucose (t/2 = 7.1). Thus, it appears that administration of hypertonic glucose may play some role in the resotration of RES function, however, for complete restoration of the RES function the combined usage of ATP-$MgCl_2$ + glucose was essential.

i) SEPSIS MODELS.

In an attempt to decrease the rapid lethal development of sepsis in our sepsis model, we have conducted additional experiments in which the cecum was punctured only once with an 18 or 22 gauge needle. The results of such experiments indicated that whereas the mortality rate within 24 hrs was 75% in rats in which the cecum was punctured twice with an 18 gauge needle, it was only 30% if the cecum was punctured only once with the same size needle. The mortality rate within 48 hrs following cecal ligation was 94% with two punctures, and 70% with one puncture with an 18 gauge needle. At the end of 5 days, however, the final mortality rates were the same, whether or not the cecum was punctured only once or twice with an 18 gauge needle. It is clear, therefore, that puncturing the cecum once, rather

than twice, with an 18 gauge needle prolongs the survival of animals, i.e. decreases the rapid development of lethality. If the cecum was punctured only once with a 22 gauge needle, instead of an 18 gauge needle, the mortality rate within 48 hrs was 46% (the corresponding mortality rate was 70% with an 18 gauge needle). The mortality rate is, therefore, lower than that observed in animals in which the cecum was punctured once with an 18 gauge needle. The mortality rate at the end of 5 days with this model was 77%. Thus, cecal ligation plus one puncture with a 22 gauge needle provides a model of slower evolving sepsis and appears to be suitable for conducting metabolic studies. We have not measured the time course of hyper- and hypodynamic circulation produced by the model in which the cecum was punctured only once with an 18 gauge needle.

4. RENAL FAILURE STUDIES

a) ACCELERATED RECOVERY OF POST-ISCHEMIC RENAL FAILURE IN RATS WITH ATP-$MgCl_2$ INFUSION.

Renal damage secondary to ischemic injury is a common and important clinical problem. Acute renal failure may occur in a number of clinical situations in which the kidney is ischemic, and commonly occurs following hemorrhagic shock, renal artery occlusion or cadaver-donar transplantation. The degree of renal injury may be modified by a number of factors such as the duration of renal artery occlusion, hypothermia, infusion of mannitol or saline prior to injury, and perfusion of kidneys with various preservatives after removal. While a number of experimental manipulations prior to the ischemic period will limit the degree of functional impairment, no agent administered after the acute injury has been consistently effective. Since, in most instances, the clinician first encounters the patient after the acute renal injury has occurred, an agent that could ameliorate the acute insult or accelerate the recovery process would be of great interest. In addition, such an agent would be an asset to new and intricate surgical techniques and to organ preservation in cadaveric renal transplantation.

Since our previous studies have shown a protective effect of ATP-$MgCl_2$ administration in shock, this study was undertaken to determine whether ATP-$MgCl_2$ infusion after 30 minutes of renal artery occlusion has any beneficial effects on renal function. In animals who receive no infusion or only $MgCl_2$, the combination of reduced glomerular filtration rate (GFR), marked diuresis, and reperfusion of the outer cortex suggested that these animals were in the early recovery phase of acute renal failure. In animals who received ATP-$MgCl_2$, there was improved GFR, no diuresis and a normal pattern of cortical blood flow distribution. These findings would suggest that infusion of ATP-$MgCl_2$ appears to have either ameliorated the effect of renal ischemia, or to have accelerated the recovery process. These observations may have important implications for future use of organ preservation and the management of acute renal failure.

In additional studies, male Sprague Dawley rats (200-300gm) were subjected to 60 min of renal ischemia by placing a clamp across the aorta proximal to the left artery and a sling around the right renal artery.

After removing the vascular clamp, one group (n = 10) received no infusion and the other group (n = 12) was infused with 25 μmoles of ATP + 25 μmoles of $MgCl_2$ IV over 30 min. Twenty-four hours later, the group which received no infusion had: 1) marked reduced glomerular filtration rate (GFR 144 ± 45μl/min/100gm, B.W.; control 917 ± 40,); 2) diminished renal blood flow (RBF 2999 ± 401μl/min/100gm, B.W.; control 5095 ± 270); 3) decreased urinary osmolarity (Uosm 700 ± 64 mosm/kg; control 1425 ± 138) and 4) increased fractional sodium excretion (FE_{Na} 1.33 ± 0.36%; control 0.17 ± 0.04). The animals infused with ATP-$MgCl_2$ showed marked improvement in 1) GFR 328 ± 64 ($p < 0.05$; 2) RBF 3604 ± 177 ($p < 0.05$); 3) Uosm 952 ± 55 ($p < 0.05$) and 4) FE_{Na} 0.57 ± 0.21 ($p < 0.05$). These results suggest that ATP-$MgCl_2$ accelerates renal recovery by: 1) diminishing tubular damage at the cellular level by providing energy essential for vital metabolic pathways, and 2) decreasing renal vascular resistance and preventing further ischemic injury.

In animals given no infusion following renal ischemia, EM studies showed marked vaculization, mitochondrial destruction and loss of brush borders. Rats infused with ATP-$MgCl_2$ had fewer ultrastructural changes and better preserved structures. These data indicate that ATP-$MgCl_2$, when infused after severe renal insult, significantly improved both glomerular and tubular function and preserved cellular structure. Thus, ATP-$MgCl_2$ appears to enhance recovery from severe acute renal injury.

b) THE USE OF ATP-$MgCl_2$ IN THE TREATMENT OF POST-ISCHEMIC RENAL INJURY IN MINI-PIGS.

We have also studied the effects of ATP-$MgCl_2$ in mini-pigs to determine if the protective effect of ATP-$MgCl_2$ infusion following ischemic renal injury observed in rats could be applied to man. The morphologic and physiologic characteristics of the pig kidney more closely resemble those of man than do those of most other experimental animals. Furthermore, consistent and reproducible determinations of renal function can be obtained in these animals with general anesthesia by the use of relatively small doses of tranquilizing agents that do not effect renal function.

Male miniature pigs (20-25kg) were subjected to bilateral renal artery occlusion for 60 min followed by reperfusion. The results indicate that renal blood flow (RBF) was reduced to 65% and glomerular filtration (GFR) to 40% of normal in saline-treated animals at 24 hours. Administration of ATP-$MgCl_2$ intravenously immediately after 60 min of ischemia resulted in the restoration of RBF to normal and GFR to 74% of normal, 24 hours later. Bilateral renal artery occlusion for 90 min resulted in a more severe impairment of renal function which was not improved by the administration of ATP-$MgCl_2$. ATP-$MgCl_2$ may exert its effect by improving renal blood flow through inhibition of post-ischemic intra-renal vasocontriction or possibly by enhancing restoration of intracellular adenine nucleotides. These observations may have important implications for future use in organ preservation and management of post-ischemic acute renal failure.

c) MECHANISM OF ACCELERATED RECOVERY OF POST-ISCHEMIC ACUTE RENAL FAILURE WITH ATP-$MgCl_2$ INFUSION.

The above studies have shown that infusion of ATP-$MgCl_2$ accelerates the recovery of post-ischemic renal failure in rats and mini-pigs. However, the fate and effects of such administered ATP are not known. To study this, bilateral renal artery occlusion in rats was performed for 30 min following which ^{14}C-ATP (12.5 umoles, specific gravity 1.8 mCi/mmole) along with equimolar $MgCl_2$ was infused intravenously in a total volume of 0.25ml (treated animals). Thirty min following the infusion of ATP-$MgCl_2$, a sample of blood was collected and small pieces of both kidneys and liver were removed and frozen in liquid N_2. The tissues were homogenized in a TCA-HCl mixture and centrifuged. Tissue extracts and deproteinized plasma samples were subjected to electrophoresis in order to separate and measure labeled nucleotides. The intracellular concentration of ATP was calculated by subtracting the counts present in the plasma from the tissue contents. The results indicated that in normal rats, ATP uptake by liver, left and right kidney, was proportionally the same. However, following bilateral renal artery occlusion, ATP uptake by kidneys was inhanced two-fold with the corresponding decrease in ATP uptake by liver. These results indicate that ischemic organs selectively take up more of the infused ATP. The fact that there was a decrease in ATP uptake by liver (which was not made ischemic) might suggest that ischemic organs trigger some signal which directs more ATP to be transported to such organs. Thus, the accelerated recovery of post-ischemic acute renal failure by ATP-$MgCl_2$ infusion could be due to provision of energy to ischemic kidneys thereby decreasing the severity of the necrotic lesion.

d) ACCELERATED RECOVERY OF ACUTE RENAL FAILURE BY INFUSION OF ADENINE NUCLEOTIDES - $MgCl_2$.

Our studies have shown that ATP-$MgCl_2$ infusion following the ischemic insult will successfully ameliorate the recovery of post-ischemic acute renal failure. In the present study, other adenine nucleotides (ADP and AMP) together with $MgCl_2$ were infused after 30 min of bilateral renal artery occlusion. Twenty-four hrs later: 1) rats that had received no infusion, ADP alone or only $MgCl_2$ had reduced GFR (355 + 40 μl/min/100 gm B.W. vs 917 + 36, control $p < 0.01$), FE_{Na} (0.65 + 0.10% vs 0.17 + 0.04 for control, $p < 0.01$), decreased RBF (3550 + 205 μl/min/100 gm B.W. vs 5095 + 171 control value), and diminished Uosm (862 + 110 vs 1425 + 132 control value; 2) rats given dopamine or phenoxygenzamine maintained low GFR (365 + 50) despite improved RBF (4678 + 222); 3) rats infused with either ADP- or AMP-$MgCl_2$ had marked improved GFR (596 + 46, $p < 0.01$), increased RBF (4269 + 223, $p < 0.01$), normalized FE_{Na} (0.18 + 0.07%, $p < 0.01$) and improved Uosm (1201 + 106, $p < 0.05$). In animals given no infusion or only $MgCl_2$, ultrastructural studies demonstrated focal cellular necrosis and marked generalized tubular cell and mitochondrial swelling, whereas rats infused with ATP-$MgCl_2$ had fewer ultrastructural changes with better preservation of cellular morphology.

The data indicate that adenine nucleotides (ATP, ADP or AMP) together with $MgCl_2$, when infused after an acute renal insult, significantly improved both glomerular and tubular function and suggest that these agents may effectively accelerate the recovery following acute renal insult.

e) DOSE RESPONSE RELATIONSHIP OF ATP-$MgCl_2$ ADMINISTRATION AFTER THE ISCHEMIC INSULT.

Studies from our laboratory has shown that infusion of 12.5umoles of ATP-$MgCl_2$ after 30 min of renal ischemia markedly accelerates the recovery from this renal injury. This study was designed to determine whether infusion of higher concentrations of ATP-$MgCl_2$ would be more effective in accelerating the recovery of renal function following ischemia

Male Sprague Dowley rats (200-300gm) were subjected to 30 min of renal ischemia by placing a clamp across the aorta proximal to the left renal artery and a sling around the right renal artery. After removing the vascular clamp, one group received no infusion and the other groups were either given 12.5μmoles, 25μmoles or 50μmoles of ATP together with equimolar amounts of $MgCl_2$, IV, over 30 min. The results indicated that the accelerated renal recovery by ATP-$MgCl_2$ was concentration-dependent and that optimal effects were observed with 50 μmoles of ATP - 50 μmoles of $MgCl_2$.

f) AMELIORATION OF TOXIC ACUTE RENAL FAILURE BY INFUSION OF ATP-$MgCl_2$.

The above studies have shown that the infusion of ATP-$MgCl_2$ immediately after 30-60 min of renal ischemia will enhance the recovery of renal failure. In this study, the effects of this agent in a model of toxic acute renal failure were examined. Rats were injected with potassium dichromate (15mg/kg, s.q.) and 24 hrs later, were infused with either 0.5ml of normal saline or 25 μmoles of ATP-$MgCl_2$.

One day after infusion: the saline-treated, dichromate-injected rats had significantly reduced C_{in} (427 ± 22μL/min/100 gm B.W. vs 1014 ± 42 control, $p < 0.01$), decreased Uosm (904 ± 82 mOsm/L vs 1654 ± 68 control, $P < 0.01$) and increased FE_{Na} (1.74 ± 0.29% vs 0.16 ± 0.03 control, $P < 0.01$). The infusion of ATP-$MgCl_2$ in dichromate-injected rats ameliorated the fall in C_{in} (670 ± 31, $p < 0.01$), increased Uosm (1190 ± 95, $p < 0.01$) and improve FE_{Na} (0.59 ± 0.14, $p < 0.01$). The ATP-$MgCl_2$ infused animals required 14 days to reach a normal GFR.

These data provide new and important information with regard to the salutary effect of ATP-$MgCl_2$ infusion and indicate that the infusion of ATP-$MgCl_2$ is effective: 1) in a model of acute renal failure which is not dependent on an initial ischemic epidode, 2) when administered 24 hrs after the initial insult and 3) in accelerating the course of recovery rather than simply modifying the severity of the initial insult.

5. HEPATIC ISCHEMIA STUDIES

a) IMPROVED HEPATIC FUNCTION AND SURVIVAL WITH ATP-$MgCl_2$ AFTER HEPATIC ISCHEMIA.

Despite its importance for liver transplantation and the problems of hepatic and multiple organ failure, little is known about the prevention or treatment of hepatic injury due to shock or ischemia. In severely injured

patients, abnormalities of hepatic function and morphology have been observed frequently. The cause or causes of these abnormalities are not established, although hepatic ischemia has been implicated. Previously we have shown hepatic mitochondrial function as well as cell membrane transport of sodium and potassium are depressed during early hemorrhagic shock, indicating the susceptibility of liver to even small insults. Because of the high metabolic rate, the hepatic cells are vulnerable to the deleterious influence of anoxia; however, the cause of cell death in the ischemic liver is not yet clear.

Previous work from our laboratory has shown that ATP levels in the liver decreased during shock and that infusion of ATP-$MgCl_2$ at the end of the shock period restored cellular ATP levels and proved beneficial in the treatment of shock. We have also shown that ATP uptake by hypoxic organs is greater than in control organs. Moreover, recent studies from our laboratory have shown that ATP-$MgCl_2$ accelerated the recovery of post-ischemic acute renal failure. Since infusion of ATP-$MgCl_2$ accelerated the recovery of post-ischemic acute renal failure and proved beneficial in the treatment of shock, the present study was undertaken to determine whether infusion of ATP-$MgCl_2$ following a period of hepatic ischemia would have any beneficial effects on the recovery of hepatic function.

In order to produce a total hepatic ischemia in rats, the portal vein as well as the hepatic artery and the bile duct was occluded by placing a tourniquet around the vessels. Colateral vessels other than the above two blood vessels to the liver were sought, and if found they were ligated. Hepatic ischemia was produced for 60 or 90 minutes. A temporary spleno-femoral venous shunt was established during the occlusion. At the end of the ischemic period, the tourniquet around the portal vein, hepatic artery and bile duct was removed in order to re-establish the blood flow to the liver. The abdominal incision then was closed in two layers and the animals received intravenously either: 1) 0.25ml of saline (controls); 2) 0.25ml of ATP-$MgCl_2$ (12.5 μmoles each); or 3) 0.25ml of ATP or $MgCl_2$ alone (12.5 μmoles each). Survival was measured over a period of five days. The survival rate in 60 and 90 minutes of hapatic ischemia series was 87.5% and 69.2% in the ATP-$MgCl_2$ group, 43.8% and 23.1% in the control group, respectively. When ATP or $MgCl_2$ alone was given after 60 minutes of ischemia, the survival rate was 20% and 30%, respectively. Thus, treatment of rats with ATP-$MgCl_2$ but not with ATP or $MgCl_2$ alone following 60 or 90 minutes of hepatic ischemia had a salutary effect on the survival of animals.

In searching for the mechanism of the beneficial effect of ATP-$MgCl_2$ following hepatic ischemia, we have measured serum enzymes and hepatic ATP levels one hour following the release of 60 minutes of ischemia. SGOT and SGPT levels (IU/ml, mean + S.E.) were 738 + 113 and 552 + 152 in the ATP-$MgCl_2$ treated group, 1981 + 179 and 1141 + 110 in the non-treated group, respectively (SGOT and SGPT normal values 38 + 4 and 12 + 2). Therefore, SGOT and SGPT levels were significantly lowered with ATP-$MgCl_2$ treatment ($p < 0.01$). Hepatic cellular ATP levels (μmoles/gm) were 1.87 ± 0.01 and 1.20 ± 0.11 ($p < 0.01$) in the ATP-$MgCl_2$ treated animals and non-treated rats, respectively (control liver ATP = 2.47 ± 0.08). Thus,

increased survival and improved hepatic function after ischemia was associated with elevated cellular ATP levels following ATP-$MgCl_2$ administration. The beneficial effect of ATP-$MgCl_2$ following hepatic ischemia could be due to: 1) provision of energy directly to hepatocytes; 2) restoration of hepatocyte function, particularly RES function; and 3) restoration of hepatic circulation and prevention of cell swelling. While the precise mechanism of action of ATP-$MgCl_2$ remains unknown, these observations may have important implications for future use in organ preservation, management of post-ischemic hepatic failure and multiple organ failure. To the best of our knowledge, this is the first demonstration that an agent has proved successful in the treatment of post-ischemic hepatic failure.

b) LIVER ULTRASTRUCTURE WITH ATP-$MgCl_2$ AFTER HEPATIC ISCHEMIA.

To determine if ATP-$MgCl_2$ has any beneficial effect on liver ultrastructure following ischemia and reflow, total hepatic ischemia in rats was produced for one hour. The liver was then fixed in situ either: 1) immediately following hepatic ischemia (ischemic group), 2) one hour following hepatic ischemia (non-treated) or 3) one hour following hepatic ischemia and ATP-$MgCl_2$ infusion (treated). Liver fixation was done by perfusing a mixture of glutaraldehyde-parafinaldehyde (2.4%) via the descending aorta. Electron micrographs of the liver from the ischemic group showed swollen mitochondria, lack of cristea, dilatation of cytoplasmic membrane and distended endoplasmic reticulum. Photomicrographs of non-treated group showed similar ultrastructural changes to the ischemic group, however, the severity of the changes was less. Hepatocytes from the ATP-$MgCl_2$ treated group showed near normal mitochondria, normal endoplasmic reticulum and cytoplasmic membrane. Our studies have also shown that liver ATP levels of non-treated group were approximately 50% of normal. Treatment resulted in elevated cellular ATP levels. Thus, the beneficial effect of ATP-$MgCl_2$ following hepatic ischemia could be due to provision of energy to hepatocytes thereby improving the microcirculation. This in turn could reverse the ultrastructural changes produced during ischemia.

c) RETICULOENDOTHELIAL SYSTEM (RES) FUNCTION FOLLOWING HEPATIC ISCHEMIA AND ITS RESTORATION WITH ATP-$MgCl_2$ ADMINISTRATION.

The above studies showed that infusion of ATP-$MgCl_2$ following 60 minutes of hepatic ischemia proved beneficial for the survival of animals. However, it is not known whether the depression in RES function following hepatic ischemia is affected by administration of ATP-$MgCl_2$. To determine this, total hepatic ischemia was produced in rats by ligation of the hepatic artery, portal vein, and the common bile duct. Splenectomy was performed following which a temporary extra-corporeal portafemoral shunt was established and maintained throughout the ischemia. At the end of the ischemic period (60 minutes), the ligature was removed, re-establishing blood flow to the liver. The animals then received IV either 0.25 ml saline (non-treated) or 0.25 ml ATP-$MgCl_2$ (12.5 μmoles each)(treated). Three hours following the end of the ischemia, RES function was evaluated by measuring the intravascular clearance of a ^{131}I-triolein labeled gelatinized test lipid emulsion. The intravascular half-time (t/2)(mean $\pm$

S.E. of eight animals in each group) in sham operated, non-treated and treated animals was 13.5 ± 0.6, 21.4 ± 2.0 and 13.2 ± 0.6 minutes, respectively. Since the t/2 in the non-treated animals was increased approximately by 60%, it indicates that the significant depression in RES function was evident even at 3 hours after ischemia. Administration of ATP-$MgCl_2$ following hepatic ischemia, however, resulted in t/2 values which were similar to sham-operated animals, indicating that ischemia induced depression in RES function was reversed with ATP-$MgCl_2$. Further studies will help to determine the mechanism by which ATP-$MgCl_2$ restores the depressed RES function.

d) HEPATOCELLULAR FUNCTION FOLLOWING HEPATIC ISCHEMIA.

Studies from our laboratory have shown that serum GOT and GPT levels were significantly increased following hepatic ischemia and that these levels were significantly decreased following administration of ATP-$MgCl_2$. However, we do not know whether hepatic function is altered much earlier than serum enzyme levels and whether ATP-$MgCl_2$ has any effect on it. We therefore measured the clearance of indocyanine green in normal rats and the t/2 values were found to be approximately 5 min. Since our ultimate purpose was to determine the indocyanine clearance following hepatic ischemia and treatment with ATP-$MgCl_2$ and since such animals would have to be splenectomized, we initially studied the effect of splenectomy on the clearance of indocyanine green. These clearances were measured at 0, 2 hrs, 3 hrs, 4 hrs, one day, two days, three days and six days following splenectomy. The results indicated that the clearance rate and, therefore, hepatic function as measured by ICG clearance, was not affected immediately following splenectomy or even after a prolonged period of time such as six days.

The clearance of indocyanine green (ICG) in normal rats was 5 min (t/2) as mentioned above. However, following hepatic ischemia, the clearance rates increased to over 10 min. Thus, hepatocellular function is also markedly altered following ischemia. We were unable to determine within this contract period whether treatment with ATP-$MgCl_2$ following hepatic ischemia has any beneficial effect on hepatocellular function.

6. SPLENECTOMY STUDIES

a) TIME COURSE OF RES DEPRESSION FOLLOWING SPLENECTOMY.

The spleen alone or in combination with other viscera, is the most frequently injured organ following blunt trauma to the abdomen. Splenectomy has usually been the recommended treatment regardless of the type or extent of splenic injury. It is well known that splenectomy causes immunological impairment and increases susceptibility to infection, however, the time course of RES depression as well as alterations in other organ functions following splenectomy is not known. To determine this, rats were splenectomized and RE function evaluated at various intervals following splenectomy and by measuring the intravascular clearance of ^{131}I-triolein gelatinized lipid emulsion. The intravascular clearance (t/2, minutes) in sham-operated rats was 7.6 ± 0.6. The t/2 at 1, 2, 4, 8,

22, 46 and 66 hours following splenectomy was 6.2 + 0.3, 7.8 + 0.8, 12.8 + 0.8, 9.6 + 0.6, 8.0 + 0.5, 6.9 + 0.6, and 8.6 + 0.7, respectively. The liver and lung % uptake of the injected emulsion in sham-operated animals was 51.3 + 1.8 and 1.8 + 0.1, respectively. Hepatic uptake was 51.4 + 2.0, 39.2 + 4.4, 27.8 + 3.4, 40.9 + 1.6, 34.4 + 2.2, 46.6 + 4.1 and 44.8 + 2.6, respectively at the above mentioned intervals following splenectomy. The corresponding lung uptake was 2.7 + 0.7, 3.0 + 0.9, 3.0 + 0.6, 14.7 + 2.2, 20.8 + 3.6, 8.0 + 1.6 and 8.9 + 2.0, respectively. These results indicate that there is a marked depression in the RES function 4 hours following splenectomy. This was reflected by increased t/2, decreased hepatic and increased lung uptake of the emulsion. Twenty-two hours after splenectomy, although the t/2 appeared normal, the lung uptake increased by 1058% and hepatic uptake was 33% lower than controls. Forty-six hours following splenectomy the lung uptake was still 344% higher even though hepatic uptake was normal. These results suggest that splenectomy may not only have deleterious effects in terms of host defense systems but also cause prolonged pulmonary changes which may jeopardize the animal as well.

b) EFFECT OF SPLENECTOMY ON SURVIVAL FOLLOWING SEPSIS.

Since the studies described above indicated that RES function in animals was depressed following splenectomy, we studied the effect of splenectomy on the survival of animals following sepsis. Sepsis in splenectomized rats was produced by cecal ligation and puncture. Saline (3ml/100gm B.W.) was given subcutaneously at that time. Ten or 16 hours (early or late sepsis, respectively) following cecal ligation and puncture, the gangrenous cecum was removed, the peritoneal cavity irrigated with warm saline and the abdominal incision closed. Saline (3.75ml) was given intravenously at that time and survival was measured over a period of 5 days. The results indicated that all non-splenectomized early septic rats survived the septic insult. However, the mortality rate was 57.1% in splenectomized animals subjected to early sepsis. Likewise, the mortality rate in non-splenectomized late septic rats was 55% and following splenectomy, it increased to 85%. Thus, splenectomizing the animals prior to cecal ligation and puncture increases the mortality of animals. These results suggest that the spleen plays an important role in the survival of animals following sepsis.

c) IMPROVED RETICULOENDOTHELIAL FUNCTION AND INITIAL SURVIVAL FOLLOWING SEPSIS WITH AUTOTRANSPLANTED SPLENIC TISSUE (AST).

After splenectomy (SPLNX), pulmonary macrophage retention of particulate matter is elevated and there is an increased risk of fatal sepsis due to alterations in host defense. The purpose of this study was to determine whether AST could prevent the pulmonary changes following SPLNX and if survival after a septic challange could be altered. Three groups of rats were studied: Group 1 - sham, group 2 - SPLNX and group 3 - SPLNX with a 90-100mg heterotopic AST placed in an omental pocket. RES function was evaluated at intervals following the initial operation by determining intravascular clearance (t/2 in min, mean + S.E., minimum of 8 rats/group) of ^{131}I-lipid emulsion. Organ retention of emulsion was expressed as % injected dose per total organ. The t/2 in group 1 was 7.6 + 0.6, in group 2 was 8.4 + 0.8, 7.9 + 0.9 and 6.8 + 0.6 and in group 3 was

6.0 ± 0.7, 3.7 ± 1.1 and 4.2 ± 0.4 at 6, 8 and 28 days, respectively. (Group 3, $p < 0.05$ compared to groups 1 and 2 except at 6 days N.S. from group 1). The pulmonary lipid retention in group 1 was 1.7 ± 0.1, in group 2 was 21.1 ± 2.7, 17.0 ± 3.5, 8.6 ± 1.9 and 3.1 ± 0.7, in group 3 was 3.1 ± 0.8, 0.4 ± 0.04, 1.5 ± 0.2 and 0.8 ± 0.2 at 6, 8, 14 and 28 days, respectively. (Group 3, $p < 0.01$ compared to group 2, N.S. from group 1). Survival with sepsis was studied in other animals in groups 1, 2 and 3 in which at 14 or 28 days after the original operation the cecum was ligated and punctured (CLP) and then removed 16 hrs later. Twenty-four hrs after cecal removal, survival rates in the three 14-day interval groups were 75% (15/20), 50% (4/8), and 100% (10/10), respectively. In the 14- and 28-day series, final survival rates 5 days following CLP and removal in group 1 were 45% (9/20), group 2 were 50% (4/8), 28.6% (2/7) and in group 3 were 60% (6/10) and 37.5% (3/8), respectively. These results indicate that AST improved t/2 of particulate matter and restored pulmonary macrophage function to normal. In addition, AST improved the initial survival following sepsis during which treatment could be instituted. Enhanced phagocytosis and improved survival with AST may be due to a RES promoter substance, such as tuftsin.

7. SPECIFICITY OF INSULIN-INDUCED MEMBRANE CONFORMATIONAL CHANGE - A SPIN LABEL STUDY

Insulin binding specificity and the nature of its binding induced membrane conformational change have been studied with two covalent spin labels on the isolated plasma membrane (PM) and sarcoplasmic reticulum (SR) membrane from guinea pig skeletal muscle. The effect of trypsin has also been investigated and compared with that of insulin. With high specific activity membrane preparations, there were two types of sites present on both PM and SR in terms of reactivity to N-(1-oxyl-2,2,6,6,-tetramethyl-4 piperidinyl)-maleimide (MSL). The highly reactive sites were in a more buried environment while the less reactive sites were more exposed. The addition of insulin to membranes labeled with low spin labeled concentrations (0.05mM) causes specific reduction of the spin label signal intensity at the PM buried site but not at the SR membrane. When membranes were labeled at high MSL concentration (0.1mM for PM and 0.6mM for SR membranes) both sites were labeled with peripheral sites predominant. The addition of insulin caused a dramatic immobilization of the label on the peripheral site on both PM and SR membrane. However, when N-(oxyl-2,2,6,6,-tetramethyl-4-piperidinyl)-bromoacedamide (BrSL) was used as a labeling agent, insulin produced no change in the environment of the BrSL sites on either PM or SR membranes. Therefore, binding of insulin appears to affect specifically the conformation and environment of the peripheral MSL binding sites. In the case of highly reactive buried sites, the insulin effect was specific to PM. This effect differed from trypsin (2ug - 2mg), which caused fragmentation of the membrane proteins as indicated by the release of the spin labeled protein fragments. It is proposed that the activation of glucose transport by trypsin takes place via the unmasking of the transport system through its proteolytic action while insulin exerts its metabolic effect through membrane conformation alterations.

8. EFFECT OF PROLONGED STARVATION ON SURVIVAL FOLLOWING TRAUMA

This study is designed to determine the effects of prolonged starvation on various parameters and on the survival of animals following trauma. Rats were fasted for 5-1/2 days (water allowed ad lib) after which a 2 cm midline incision was made and the cecum ligated. Saline (3ml/100gm B.W.) was given subcutaneously at that time and again at 10,17, and 34 hours following ligation (1,1.5 and 2.5ml/100gm B.W., respectively). Food was allowed 36 hours after cecal ligation (CL) and survival was measured over a period of five days. The mortality rate in these animals was 60% (12/20) compared to 0% (0/15) in twenty-four hours fasted animals. Starvation alone for seven days did not produce any mortality. In an additional study, reticuloendothelial (RE) function was evaluated 10 hours following cecal ligation by measuring the intravascular clearance of ^{131}I-triolein labeled gelatinized lipid emulsion. There was no significant difference in the clearance rates of emulsion in various groups suggesting that the phagocytic function was not impaired by starvation plus cecal ligation. However, hepatic and splenic uptake of emulsion decreased by 26% and 74%, respectively following starvation plus cecal ligation and associated with this was 738% increase in retention of the emulsion by the lung. The hemotcrit in these animals was 61% (normal value 46). The serum levels of γ-globulin (gm %) in 24 hours fasted, six days fasted and six days starved plus cecal ligated rats were: 0.49 ± 0.02, 0.90 ± 0.08 and 0.33 ± 0.04, respectively. The finding of increased γ-globulin levels during starvation was quite unexpected. Serum GOT levels were not affected by prolonged starvation alone, however, starvation plus cecal ligation results in its elevation 31 ± 9 vs 85 ± 10 IU/ml. Preliminary experiments also indicate that hepatic and renal ATP levels decreased from 2.6 ± 0.5 and 1.8 ± 0.4 to 1.6 ± 0.1 and 1.2 ± 0.2 μmoles/g, respectively, following starvation plus cecal ligation. These results indicate that increased metabolic demand following trauma (i.e., cecal ligation), coupled with severe caloric deprivation may have an effect on RES function since splenic lipid emulsion uptake decreased following starvation plus cecal ligation. The relationship of these findings to immune function remains to be determined. Alghough the precise cause of mortality is not known, altered hepatic, pulmonary and RES function may play a role.

9. LOCAL EFFECTS OF THERMAL INJURY ON SKELETAL MUSCLE, BLOOD FLOW AND NUCLEOTIDE LEVELS.

Previous work has shown that 3 days following a 3 second scald of one hind limb, in vitro glucose utilization was markedly increased in soleus muscle from the burned limb but not in soleus from the contralateral unburned limb. The aim of the present study was to evaluate factors which might contribute to this local metabolic alteration. Three days following a 3 second scald of one hind limb of the rat, blood flow through soleus and gastrocnemius muscles of the burned limb as measured with labeled microspheres was increased 167% ($p < 0.01$) and 58% ($p < 0.001$), respectively. Calf muscles of the burned limb, frozen in situ showed a 45% decrease in ATP ($p < 0.001$), 37% decrease in ADP ($p < 0.01$), 132% increase in pyruvate ($p < 0.001$) and 377% increase in lactate ($p < 0.001$). Blood flow and ATP, ADP total nucleotides and pyruvate levels of calf muscles of unburned limb of burned rats did not differ from controls, but AMP and lactate were increased 115% ($p < 0.05$) and 144% ($p < 0.001$), respectively. The

decrease in ATP and increase in AMP and lactate in muscles of the burned limb suggest an increased rate of glycolysis in vivo which may be due, in part, to the stimulation of phosphofructokinase. Furthermore, the increased AMP and lactate may contribute to the increase in blood flow in muscles of the burned limb. It is proposed that thermal injury alters local adenine nucleotide levels which is associated with elevated glucose utilization and blood flow in muscles of the burned region.

10. EARLY DETECTION OF ISCHEMIC DAMAGE IN THE SMALL INTESTINE

Two functional parameters, transmural potential difference (TPD) and stimulated muscle contractions, were used to detect ischemic damage to the mucosa and the musculature, respectively, of distal jejunal segments. Bowel ischemia in rats was produced by doubly ligating the arterial and venous branch supplying that segment of the jejunem as well as the proximal and distal bowel lumen. In the first study, mucosal TPD was recorded by placing catheter probe electrodes (5% Agar in lactated Ringer's) both intraluminally and intraperitoneally. An exponential decline in the TPD (mVolts), plateauing at 15 min and with T/2 = 8 min was observed. At times of T = 0, 5 min, 10 min, 15 min, 30 min, 1 hr and 2 hrs, the TPD (mean + S.E. of 5 rats) were 6.8 + 0.7, 4.6 + 0.7, 3.2 + 0.5, 2.4 + 0.2, 2.1 + 0.5, 1.7 + 0.6 and 1.0 + 0, respectively (compared to T = 0, $p < 0.005$ at 10 min and $p < 0.001$ at all subsequent times). Microscopically, after 15 min of ischemia, the mucosal epithelium was minimally separated from the tips of the underlying villi. In the second study, the contractile response of the jejunal circular muscle to electrical stimulation (70 volts, 100 Hz/sec, 5 msec pulses for 5 sec) was recorded from isolated cross sectioned strips, 1mm in width, mounted in a perfusion chamber. The strips were continuously perfused and oxygenated at 37°C. With a preload of 0.5g, the strips showed intact muscular contractions at T = 0, 30 min, 1 hr, 12 hrs, 14 hrs, 18 hrs and 22 hrs. The number of positive contractions/number of samples tested (3 samples/rat) at the above-mentioned times were: 3/3, 3/3, 3/3, 5/6, 3/3, 6/6 and 5/6, respectively. Histologically, after 22 hrs of ischemia, the mucosa had completely sloughed but the muscle appeared intact.

The results presented here indicate that transmural potential difference measurements provide an extremely sensitive indication of early minimal mucosal damage. In contrast, muscle contractions in response to electrical stimulation persist despite prolonged (22 hrs) ischemic insult.

11. CHARACTERIZATION OF A PROBABLE ATP TRANSPORT SYSTEM.

Since our pioneer studies concerning ATP transport into the cell have now been well documented by other investigators, including investigators from the NIH, we have begun conducting studies which we hope will characterize the system which transports ATP into the cell. Our preliminary results have shown that addition of 10^{-3}M ouabain to the incubation medium inhibited ATP uptake process by 70%; moreover, insulin has been shown to stimulate the Na, K-ATPase activity stimulated ATP uptake process by more than 60%. These preliminary results strongly suggest that ATP could be transported into the cell by the sodium pump, i.e., the Na, K-ATPase since the ATP uptake process responded both to the stimulatory effects of insulin and inhibitory effects of ouabain.

Our preliminary results have also shown that various metabolic poisons (e.g. silver nitrate, copper sulphate, zinc acetate, sodium salicylate and dinitrophonol) also inhibited the ATP uptake process. These results may very well indicate that ATP uptake is an active process and that it requires mitochondrially produced ATP for its operation.

12. HEMODYNAMIC RESPONSES TO ATP-$MgCl_2$ INFUSION.

a) HEMODYNAMIC (DOSE-RESPONSE) EFFECT OF ATP-$MgCl_2$ INFUSION IN ANESTHETIZED NORMAL DOGS.

Intravenous administration of ATP-$MgCl_2$ after hemorrhagic shock, sepsis, burns, endotoxin shock and post-ischemic hepatic failure increases survival in animal models. The specific effects of this complex on myocardial performance and the differences in action between the right atrial (RA) and left atrial (LA) infusion are not known, however. This study was undertaken to examine the dose-repsonse effects of ATP-$MgCl_2$ infusion on cardiac and systemic hemodynamics in normal anesthetized dogs.

Ten adult mongrel dogs (median weight 19.5kg) were anesthetized with pentobarbital (30mg/kg). The heart was exposed through a median sternotomy, placed in a pericardial sling and cannulated to measure aortic root pressure (AOP), left ventricular pressure (LVP), LVP dP/dT, left atrial pressure (LAP) and heart rate. Cardiac output (CO) was measured by thermodilution. ATP-$MgCl_2$ was infused either via the LA or RA in three dose rate ranges (mg/kg/min): low-range (LR), 0.01 to 0.10; mid-range (MR), 0.10 to 1.0; and high-range (HR), 1.0 to 10.0. The order and route of administration were randomized and hemodynamic parameters were allowed to return to stable baseline values between each infusion. Stroke volume (SV), stroke work index (SWI) and systemic vascular resistance (SVR) were calculated from the measured data. Responses (except SVR) were expressed as percent change from the previous baseline.

The results indicated that infusion of ATP-$MgCl_2$ increased CO by a maximum of 30.5 $\pm$ 4.5% ($p < 0.01$) in the LR with LA infusion and by a maximum of 28.3 $\pm$ 6.8% ($p < 0.01$) in the MR with RA infusion. CO decreased at dose rates greater than 0.63 and 1.30 for LA and RA infusion, respectively. LVP dP/dT increased with RA infusion in the LR and MR by a maximum of 13.0 $\pm$ 2.5% ($p < 0.01$) but decreased with LA infusion in all dose rate changes. SWI increased with RA infusion in the LR and MR by a maximum of 32.1 $\pm$ 3.4% ($p < 0.01$) and decreased at dose rates greater than 1.3. With LA infusion, SWI decreased in all dose rate changes. The difference between LA and RA infusion for SWI and LVP dP/dT was significant ($p < 0.01$) in all dose rate ranges. Heart rate decreased continuously throughout all ranges to a maximum decrease of -36.3 $\pm$ 6.2% ($p < 0.005$) with LA infusion and -33.1 $\pm$ 5.2% ($p < 0.01$) for RA infusion in the HR; however, the difference between the two routes was not significant. SV increased continuously throughout all ranges by a maximum of 128.0 $\pm$ 13.5% ($p < 0.005$) for LA infusion and 25.0 $\pm$ 2.8% ($p < 0.01$) for RA infusion in the HR. The LA SV response was significantly greater than the RA response ($p < 0.01$). Moreover, the increase in SV was associated with the disproportionately greater decrease in the heart rate than change in CO. The mean SVR

(mm/Hg/l/min) decreased with RA infusion from 110.0 ± 15.6 in the LR to 48.0 ± 4.8 ($p < 0.005$) in HR. SVR also decreased with LA infusion from 67.2 ± 9.0 in the LR to 22.2 ± 7.8 ($p < 0.005$) in the HR. The two curves were parallel and significantly different throughout all ranges ($p < 0.01$).

These results indicate that infusion of ATP-$MgCl_2$ via the RA in the LR and the MR significantly increased CO, LVP dP/dT and SWI; however, LA infusion decreased both LVP dP/dT and SWI in all ranges. Thus, when infused via the RA, ATP-$MgCl_2$ acts as a positive inotrope at dose rates less than 1.3mg/kg/min but not when infused via the LA. The consistent decrease in heart rate throughout all dose ranges by both LA and RA infusion as well as the disproportionately greater decrease in heart rate than in CO implies that ATP-$MgCl_2$ is also a negative chronotrope. The consistent and parallel decrease in SVR with both LA and RA infusion demonstrates that ATP-$MgCl_2$ is also a systemic vasodilator. The different effects of ATP-$MgCl_2$ when infused via the RA vs the LA suggests that ATP-$MgCl_2$ undergoes an alteration in its passage through the pulmonary vasculature.

Infusion of ATP-$MgCl_2$ via the right side of the heart therefore produces three major salutary effects on myocardial function: positive inotropic, at dose rates less than 1.3mg/kg/min; negative chronotropic, at dose rates 0.01 to 10.0mlg/kg/min; and systemic vasodilatory, at dose rates 0.01 to 10.0mg/kg/min. The positive inotropic effects of ATP-$MgCl_2$ were not observed when the complex was infused via the LA rather than the RA. ATP-$MgCl_2$, therefore, appears to be altered in its passage through the pulmonary vascular bed to yield different hemodynamic effects with RA vs LA infusion. The positive inotropic and negative chronotropic effects and peripheral vasodilatory actions of ATP-$MgCl_2$ suggest a potential clinical applicability for the complex in low output states.

Regional blood flow measurements using labeled microspheres with and without ATP-$MgCl_2$ infusion were not conducted in these animals due to lack of funds.

b) HEMODYNAMIC (DOSE-RESPONSE) EFFECTS OF ATP-$MgCl_2$ INFUSION IN ANESTHETIZED HYPOVOLEMIC DOGS.

Since ATP-$MgCl_2$ infusion in the normal anesthetized, open-chest dog produces positive inotropic, negative chronotropic and peripheral vasodilatory effects, this study was undertaken to determine the effects of ATP-$MgCl_2$ during hypovolemic conditions (H). To study this, ten anesthetized, open-chest dogs were bled to a mean pressure of 81mmHg, producing: a decrease in cardiac output (C) (L/min) from 1.97 ± 0.60 in the control state (C) to 1.06 ± 0.33 in the H state ($p < 0.01$); a decrease in mean aortic root pressure (AOP)(mmHg) of 126.1 ± 11.1 (C) to 81.1 ± 9.5 (H) ($p < 0.01$); a decrease in LVP dP/dT (mm/Hg/sec) from 2605 ± 549 (C) to 1845 ± 398 (H)($p < 0.01$); a decrease in stroke volume (SV)(ml) from 12.1 ± 3.6 to 6.1 ± 1.5 (S)($p < 0.01$); a decrease in left ventricular stroke work index (SWI)(gm.m/kg x 10^{-3}) from 641 ± 147 (C) to 249 ± 129 (H)($p < 0.01$); and an increase in systemic vascular resistance (SVR)(mm/Hg/ml/min x 10^{-3}) from 63.9 ± 15.5 (C) to 80.9 ± 20.0 (H)($p < 0.01$). Following stable H, ATP-$MgCl_2$ was infused via a catheter in the right atrium in three dose

rate ranges (mg/kg/min)-low range (LR) 0.01-0.10, mid-range (MR) 0.10-1.0, high range (HR) 1.0-10.0. The maximum increase in CO, expressed as percent change from the previous baseline, was 16.5 + 7.8% in MR and decreased in HR. LVP dP/dT increased by a maximum of 22.4 + 12.1% in MR and decreased in HR. In addition, SWI increased by a maximum of 15.4 + 7.2% in MR and decreased in HR. Therefore, ATP-$MgCl_2$ produced a positive inotropic effect in hypovolemia at a dose rate less than 1mg/kg/min. SV increased continuously throughout all ranges by a maximum of +79.3 + 39.3% in HR and was associated with decreased heart rate throughout all ranges by a maximum of -40.9 + 8.5% in HR. Thus, ATP-$MgCl_2$ is also a negative chronotrope. Aortic root pressure increased with the increase in CO despite the decrease in SVR throughout all dose ranges (maximum decrease to 68.9 + 4.5 in the HR). ATP-$MgCl_2$ therefore also acted as a peripheral vasodilator. Thus, the mid-range dose of ATP-$MgCl_2$ produced positive inotropic, negative chronotropic and peripheral vasodilatory effects during hypovolemia. Since these effects restored hemodynamic parameters towards normal, ATP-$MgCl_2$ could have potential clinical applicability even during hypovolemia.

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